Biogeochemistry of Methane in a Shallow Sandy Aquifer

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Ph. d. Dissertation

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Department of Geology and Geotechnical Engineering
Technical University of Denmark
and
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Radius

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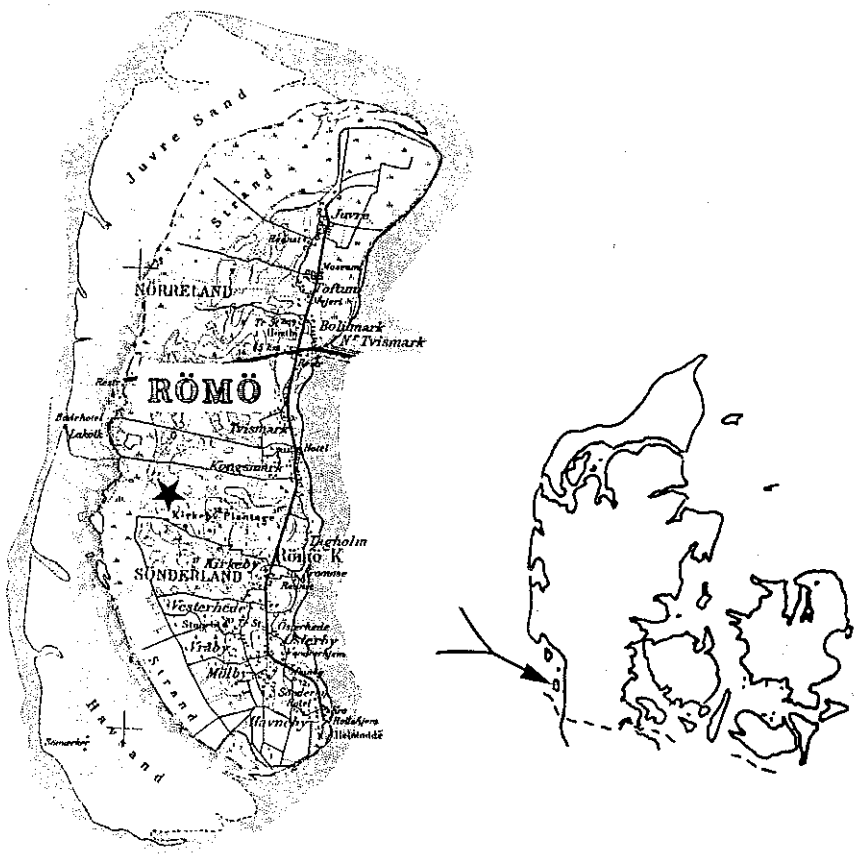

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I. Project background

This ph.d. project, entitled "Biogeochemistry of methane in a shallow sandy aquifer" was undertaken under the Strategical Environmental Programme, Subprogramme 2 - Groundwater (1992-1996). The project is a subproject of the project "Generation of Sulfate and Methane in Groundwater". The Danish Research Academy has administrated the ph.d. project in association with the Geological Survey of Denmark and Greenland, Ministry of Environment and Energy and with the Department of Geology and Geotechnical Engineering, Groundwater Research Center, Technical University of Denmark. Associate Professor Dieke Postma, Department of Geology and Geotechnical Engineering, Groundwater Research Center, Technical University of Denmark served as supervisor on the project. Associate Professor Rasmus Jakobsen, Department of Geology and Geotechnical Engineering, Groundwater Research Center, Technical University of Denmark and Senior Researcher Christian Grøn, Plant Biology and Biogeochemistry Department, RISØ National Laboratory have been cosupervisors.

The project "Generation of Sulfate and Methane in Groundwater" was carried out by a group of researchers from the University of Aarhus, Groundwater Research Center at the Technical University of Denmark, and the Geological Survey of Denmark and Greenland. Dieke Postma was the project manager on this project.

II. Acknowledgements

Many project coworkers and colleagues have contributed with a valuable help and support through the project, and I am indebted to them all. First of all, I would like to thank Dieke Postma, Rasmus Jakobsen and Christian Grøn for their inspiring supervision throughout the project. Without the ability of Ellen Zimmer Hansen to solve all sorts of technical problems, in the field as well as in the laboratory, this project would not have been possible. It would also not have been possible without the never ending willingness of Lene Jensen, Bente Frydenlund, Joan Jensen and Henrik Skov to put in their best in analysing the samples. A special thanks goes to Troels Laier for introducing me to the world of gas chromatography and to Niels Iversen for letting me in on the secrets of methane oxidation rate measurements.

I also wish to thank Christian Grøn, Rasmus Jakobsen, Ole Larsen, Ellen Zimmer, Søren Frank, Rasmus Rune Nielsen, Gustav Holmefjord, Hans Hansen, Simon Ellingsgaard, Karin Gleie and Henrik Bohn for their patient assistance, their ability to solve all sorts of problems and their pleasant company during the many hours of field work, that led to these results.

Last, but not least, I wish to thank my family for their tremendous support and assistance throughout the project and particularly in the final hectic days.

III Abstract

The data for this study are from a young (<2500 years) shallow aeolian/marine sandy aquifer on the island of Rømø, Denmark. The dissertation describes the first direct measurements of methane production and methane oxidation rates and the first detailed measurements of formate and acetate concentrations in an aquifer. Time series carried out for the rate measurements indicate that the methods are reliable.

Methane production occurs mainly via the CO_2 reduction pathway, but there is also a significant, and highly variable, contribution from acetate fermentation. The rates are 1-3 orders of magnitude lower than those previously measured in marine sediments, but similar to rates measured in some lake sediments. The rates vary considerably over depth and between sampling locations, which most likely reflects, that reactive organic matter is unevenly distributed.

Mass balance considerations show, that most of the organic matter used in the redox processes must come from the soil zone with the infiltrating water, possibly attached to colloids. This is different from deep aquifer systems, where it is usually organic matter, deposited with the sediment, that is being decomposed.

There is a distinct separation between sulfate reduction and methane production. When sulfate enters previously methanogenic parts of the aquifer, sulfate reduction replaces methane production, whereas methane is readily formed in previously sulfate reducing parts of the aquifer, when sulfate is no longer present. In contrast, sulfate reduction and Fe(III)-oxide reduction are not spatially separated. In sulfate free parts of the aquifer, another redox process, possibly Fe(III)-oxide reduction, also occurs concurrently with methane production. This redox process is able to outcompete methane production almost completely in some sulfate free parts of the aquifer, but not in others. The competitive suppression of methane production occurs concurrently for the CO_2 reduction and acetate fermentation pathways.

In contrast to the situation in marine sediments, methane oxidation coupled to sulfate reduction is not a quantitatively important process. This most likely reflects, that the concentrations of methane and sulfate are much lower than in marine sediments.

The hydrogen concentration reflects the dominating redox process to some extent, but the energy yield of CO_2 reduction is so low, that this process must take place in microniches with higher H_2 concentrations or by hydrogen transfer between juxtaposed bacteria. The H_2 concentration in the bulk sediment must therefore be controlled by another redox process, e.g. Fe(III)-oxide reduction, even when methane production is the dominating redox process. Accordingly, the H_2 concentration cannot be used to determine, which redox process dominates.

The concentrations of formate and acetate are completely unrelated to the dominating redox process. Calculations of the energy available from acetate turnover show, that competitive suppression of methane production from acetate cannot be due to a limited energy yield of the process, which is the traditional explanation. It is suggested, that the hydrogen concentration might control the turnover of acetate by causing acetogenic methanogenic bacteria to switch their metabolism from CH_4 production to H_2 production from acetate, when the H_2 concentration is low enough to make this energetically favourable.

IV Dansk resume

De data, som præsenteres i denne afhandling, er fra en ung (<2500 år) overfladenær akvifer på Rømø, Danmark. Akviferen består af æolisk og marint sand. Afhandlingen beskriver de første direkte målinger af methandannelsesrater og methanoxidationsrater samt de første detaljerede målinger af format- og acetatkoncentrationer i en akvifer. Tidsserier for ratemålingerne indikerer, at de anvendte metoder er pålidelige.

Methanproduktionen sker primært via CO_2 reduktion, men der er også et væsentligt og meget varierende bidrag fra acetatfermentation. De målte rater er 1-3 størrelsesordener mindre end dem, som tidligere er målt i marine sedimenter, men svarer til de rater, som er målt i nogle søsedimenter. Raterne varierer meget over dybde og mellem de undersøgte prøvetagningssteder, hvilket sandsynligvis skyldes, at det reaktive organiske materiale er ujævnt fordelt.

Massebalanceberegninger viser, at hovedparten af det organiske materiale, som bliver forbrugt i redoxprocesserne, kommer fra jordbunden med det infiltrerende vand, muligvis bundet til kolloider. Dette er forskelligt fra dybe akviferer, hvor det normalt er organisk stof aflejret med sedimentet, som bliver omsat.

Der er en klar adskillelse mellem sulfatreduktion og methanproduktion. Når sulfat trænger ind i tidligere methanogene dele af akviferen, erstattes methanproduktion med sulfatreduktion, hvorimod methanproduktion straks går i gang i tidligere sulfatreducerende dele af akviferen, når sulfat ikke længere er til stede. I modsætning til dette er der ingen adskillelse mellem jern(III) reduktion og sulfatreduktion. I de sulfatfrie dele af akviferen foregår en anden redox proces, muligvis jern(III) reduktion, også samtidig med methanproduktionen. Denne redoxproces er i stand til næsten helt at udkonkurrere methanproduktion i nogle sulfatfrie dele af akviferen, men ikke i andre. Udkonkurreringen af methanproduktion sker både for CO_2 reduktions og acetatfermentations dannelsesvejene.

I modsætning til situationen i marine sedimenter er methanoxidation koblet til sulfatreduktion ikke nogen kvantitativt væsentlig proces. Dette skyldes sandsynligvis, at koncentrationerne af såvel methan som sulfat er meget lavere end i de marine sedimenter.

Hydrogen koncentrationen afspejler i nogen grad den dominerende redoxproces, men energiudbyttet ved CO_2 reduktion er så lavt, at CO_2 reduktionen må foregå i mikronicher med højere hydrogen koncentrationer eller ved direkte overførsel af hydrogen mellem tætsiddende bakterier. Hydrogen koncentrationen i bulk sedimentet må derfor være styret af en anden redox proces, f.eks. jern(III) reduktion, selv når methanproduktion er den dominerende redox proces. Dette betyder, at hydrogen koncentrationen ikke kan bruges til at afgøre hvilken redox proces,

som dominerer.

Format og acetat koncentrationerne afspejler slet ikke den dominerende redoxproces. Beregninger af det energimæssige udbytte ved acetatomsætning viser, at fraværet af methanproduktion ud fra acetat i dele af akviferen ikke kan skyldes et lavt energiudbytte ved processen, som det hidtil har været antaget. Det foreslås, at hydrogen koncentrationen styrer omsætningen af acetat, ved at acetatforbrugende methanogene bakterier skifter fra at producere methan til at producere hydrogen, når hydrogen koncentrationen bliver tilstrækkeligt lav til, at dette er energimæssigt fordelagtigt.

1. Introduction

The danish water supply has traditionally been based on the utilization of water from shallow aquifers. However, the water quality in these aquifers is threatened by pollution from numerous anthropogenic sources: agricultural land use, industry etc. For this reason, an increased interest has developed in the possibility of utilizing deeper aquifer systems to secure a supply of unpolluted water. Reduced conditions are often found in such aquifers, and a need has therefore arisen to know more about, what controls the water chemistry in reduced aquifers .

This project is part of a research program focussing on geochemical processes in reduced aquifers. While methane in itself does not pose any critical problems to the water supply (it is easily removed by extended airation of the water), an understanding of the microbiologically mediated processes, that lead to methane formation in aquifers, might prove important for our ability to predict the fate of organic pollutants in groundwater.

While reduced conditions are most common in deep aquifers, practical considerations speaks strongly for choosing a shallow aquifer for a detailed geochemical study as this. Moreover, while shallow reduced aquifers are geologically and hydrogeologically different from deep aquifers in many ways (lower sediment age, shorter residence time of the water, no confining aquitards etc.), the processes are presumably the same. For this reason, the shallow Rømø aquifer was chosen for this study. The Rømø aquifer has been subject to previous detailed geochemical investigations, focussing on sulfate reduction (Jakobsen & Postma, 1994; Jakobsen, 1995) and on Fe(III)-oxide reduction (Larsen, 1998).

1.1 Decomposition of organic matter and redox processes.

There are two principle sources for organic matter in aquifers. One is organic matter deposited with the sediment, the other is organic matter leached from the soil zone by infiltrating water. The latter source seems to be particularly important in aquifers with a shallow water table, because the short residence time of water in the unsaturated zone allows more organic matter to reach the saturated zone without being decomposed (Starr, 1988).

Once present in the aquifer, organic matter can act as an electron donor for a wide range of bacterially mediated redox processes. The most common of these redox processes are O_2 reduction (aerobic respiration), NO_3^- reduction (denitrification), Mn(IV)-oxide reduction, Fe(III)-oxide reduction, SO_4^{2-} reduction and CO_2 reduction (methane production). Depending on which electron acceptor is used in the redox process, the decomposition of organic matter will be carried out by different strains of bacteria and proceed via different pathways. An overview of these pathways is shown in figure 1.1.

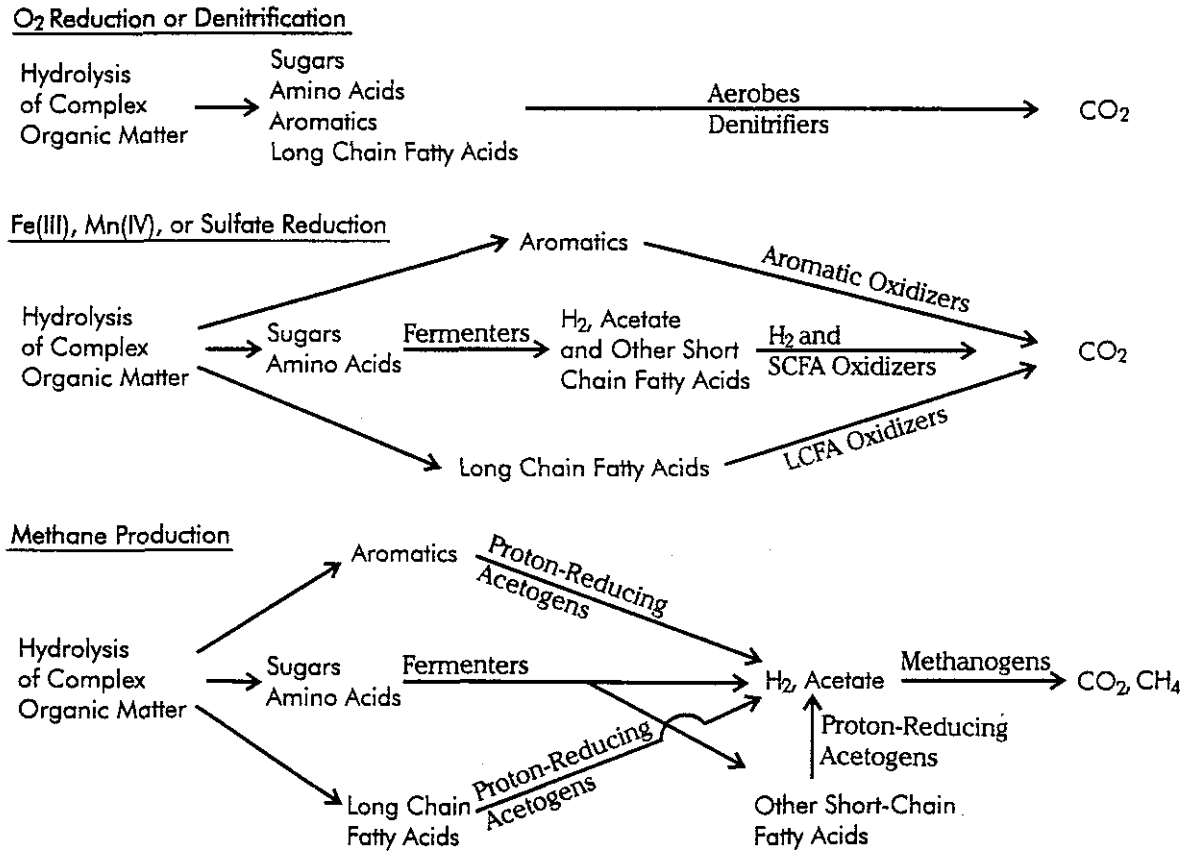

Figure 1.1. Pathways of organic matter decomposition by various microbial processes. From (Lovley & Chapelle, 1995).

The decomposition of organic matter always starts with the hydrolysis of large, complex molecules to smaller and simpler ones like sugars, amino acids, aromatics and long chain fatty acids. Aerobic bacteria can, by themselves, completely oxidize a wide variety of these simple organic molecules to CO_2, using oxygen or nitrate as the electron acceptor. Most anaerobic bacteria are, on the other hand, much more selective, and can only oxidize completely a limited number of organic molecules. A third step is therefore necessary in the decomposition of organic matter with Fe(III), Mn(IV) or sulfate as the electron acceptor, where sugars and amino acids are fermented into H_2, acetate and other short chain fatty acids by the fermenting bacteria. Methanogenic bacteria are even more selective and can only utilize a very few substrates. A fourth group of bacteria, the proton-reducing acetogens, are therefore also involved in the decomposition of organic matter in methanogenic environments.

It is often observed, that the redox processes are spatially separated in sediments, even though several possible electron acceptors are present. The explanation usually given for this is, that the energy available to microorganisms from decomposition of organic matter depends strongly on which electron acceptor in involved in the process. It is supposed then, that the electron acceptor,

that yields most energy per mole carbon oxidized, will be preferred, as long as it is present in concentrations, that does not limit the bacterial activity. Assuming standard conditions (25 °C and unimolar concentrations of all solutes involved), the energy released by oxidation of one mole carbon (with an oxidation state of zero) with the various electron acceptors are given in figure 1.2

Reaction:	Released energy (kJ/mol CH_2O)
$CH_2O + O_2 \rightarrow CO_2 + H_2O$	-475
$5CH_2O + 4NO_3^- \rightarrow 2N_2 + 4HCO_3^- + CO_2 + 3H_2O$	-448
$CH_2O + 3CO_2 + H_2O + 2MnO_2 \rightarrow 2Mn^{2+} + 4HCO_3^-$	-349
$CH_2O + 7CO_2 + 4Fe(OH)_3 \rightarrow 4Fe^{2+} + 8HCO_3^- + 3H_2O$	-114
$2CH_2O + SO_4^{2-} \rightarrow H_2S + 2HCO_3^-$	-77
$2CH_2O \rightarrow CH_4 + CO_2$	-58

Figure 1.2. Redox reactions and associated change in Gibbs Free Energy, ΔG_0, at standard conditions. From (Berner, 1980).

The sequence of redox processes, that can be predicted from figure 1.2 is, that O_2 reduction will dominate until O_2 is used up, then nitrate reduction should take over, then Mn(IV)-oxide reduction, Fe(III)-oxide reduction, sulfate reduction and finally methane production. Given of course, that these electron acceptors are present at all. As a general framework, this model seems to be valid. Other redox processes than O_2 reduction are generally not observed in sediments, where O_2 is present is significant concentrations. The presence of nitrate in a sediment generally also excludes the redox processes with lower energy yields (e.g. Starr, 1988; Frind et al. 1989). When it comes to the anaerobic redox processes; Mn(IV)-oxide reduction, Fe(III)-oxide reduction, sulfate reduction and methane production, the situation is a little more complicated. Since the decomposition of organic matter often proceeds in several steps in these processes (figure 1.1), the total energy released from oxidation of 1 mole of carbon does not provide much information about the energy available for the single microorganisms involved.

The molecules produced by fermenting bacteria (fermentation products) are usually present in sediments in concentrations, that are measured in nmol/l for H_2 and µmol/l for acetate and other short chain fatty acids. These low concentrations suggests, that one of the first two steps in the decomposition of organic matter, hydrolysis or fermentation, must be rate limiting for the overall process. Since bacteria exists, that are able to utilize H_2 and acetate with both Mn(IV), Fe(III), SO_4^{2-} and CO_2 (methane production) as electron acceptors, it has been proposed, that segregation of these redox processes is due to competition for these substrates between different microorganisms (Lovley & Klug, 1983; Lovley & Phillips, 1987; Chapelle & Lovley, 1992). The theory is, that the microorganisms gaining most energy from the oxidation of one mole of H_2 or acetate will

grow faster than those gaining less energy, and since the supply of H_2 and acetate is limited by one of the previous steps in the decomposition of organic matter, the concentrations of H_2 and acetate will become so low, that less effective microorganisms cannot be active, even though they might still be present in the sediment. This phenomenon has been termed competitive exclusion.

The concept of competitive exclusion was used by (Lovley & Goodwin, 1988) to show theoretically, and demonstrate, using data from natural sediments, how competition between different terminal electron accepting processes (TEAP`s) leads to specific levels of H_2 for each redox zone. (Lovley & Goodwin, 1988) predicted typical H_2 levels of 0.1-0.5 nM for Fe(III)-oxide reduction, 1-3 nM for sulfate reduction and 7-10 nM for methanogenesis. In subsequent studies of anaerobic aquifers, ranges of H_2 concentrations have been determined to 0.1-0.8 nM for Fe(III)-oxide reducing, 1.0-4.0 nM for sulfate reducing and 5-25 nM for methane producing aquifers (Chapelle & Lovley, 1992; Vroblesky & Chapelle, 1994; Chapelle et al. 1995). (Jakobsen, 1995) measured H_2 concentrations in the Rømø aquifer, and found, that the H_2 concentration give a poor picture of the dominant TEAP in this aquifer. In general the H_2 concentration is lower than predicted by (Lovley & Goodwin, 1988) and so low, that CO_2 reduction is not energetically favourable in parts of the aquifer. However, high concentrations of methane in parts of the aquifer indicated ongoing methanogenesis. In a later study, similar results were found in a landfill leachate plume in Grindsted, Denmark by (Jakobsen et al. 1998).

(Jakobsen, 1995; Jakobsen et al. 1998) explained the lack of correlation between H_2 concentration and TEAP by concurrent occurrence of the redox processes and also suggested, that CO_2 reduction must take place within microniches with higher H_2 concentrations or by interspecies hydrogen transfer between juxtaposed bacteria, as previously proposed by (Conrad et al. 1985; Thiele & Zeikus, 1988).

In a review of sulfate reduction and Fe(III)-oxide reduction in sediments, (Postma & Jakobsen, 1996) found that these two processes often seems to occur concurrently. An explanation for this was sought in the thermodynamics of the two processes under in situ conditions, and (Postma & Jakobsen, 1996) concluded, that concurrent reduction of Fe(III)-oxides and sulfate is thermodynamically possible under a vide range of natural conditions, depending among other things on the reactivity of Fe(III)-oxides and, in Fe^{2+} rich porewater, on the pH of the porewater. Under some conditions, sulfate reduction is more energetically favourable than reduction of less reactive Fe(III)-oxides. Accordingly, (Postma & Jakobsen, 1996) proposed a slightly different explanation for the segregation of the anaerobic redox processes, where the fermenting step is thought to be rate limiting and the electron accepting processes are considered to be close to equilibrium. Using this approach, the sequence of redox processes shown in figure 1.2 can again be predicted, but only when the in situ ΔG_r`s are significantly different. When this is not the case, the partial

equilibrium model predicts, that the redox processes might occur concurrently.

The TEAP`s do not occur at true equilibrium, since the bacteria must gain sufficient energy from the process to enable cell growth. (Hoehler, 1998) for instance reported, that CO_2 reduction in Cape Lockout Bight occurred at ΔG_r values near -10 to -15 kJ/mole H_2, whereas sulfate reduction occurred at a slightly higher energy yield (more negative ΔG_r). (Hoehler, 1998) also found, that temperature, pH and the concentration of sulfate had a large influence on the H_2 concentration, presumably due to the resulting changes in ΔG_r. This supports the hypothesis, that the H_2 concentration is thermodynamically controlled, and that the bacteria keeps it as close to equilibrium as biologically possible. However, there is no reason to expect specific H_2 concentration levels for specific TEAP`s. Rather one should expect to find a ΔG_r value for the dominating TEAP, that is close to the biologically determined threshold level. The H_2 concentration will then be controlled, not only by the dominating TEAP, but also by the temperature and the activity of the various solutes involved in the reactions, as proposed by (Postma & Jakobsen, 1996).

1.2 Methane in aquifers and methanogenic pathways

Most previous studies of methane in aquifers have focussed on whether the methane is thermogenic or bacterial in origin. A classic tool for this purpose is to look at the relation between CH_4 and longer chain hydrocarbons (C_2-C_4). Bacterially produced methane never contains significant amounts of (C_2-C_4) hydrocarbons, and a high content of these (>0.05 %) is therefore a very good indicator of a non bacterial origin of the methane (Schoell, 1980).

Another tool often used to classify methane is the relation between light and heavy isotopes of carbon and hydrogen ($^{13}C/^{12}C$ and D/H). In bacterially mediated processes, light isotopes are consumed preferentially to heavy isotopes, and accordingly bacterially formed methane is depleted in ^{13}C and D relative to the parent material: CO_2 and H_2O. Furthermore, methanogens that produce methane from H_2/CO_2 usually have larger fractionation factors for $^{13}C/^{12}C$ than those producing methane from acetate (Whiticar et al. 1986). It is therefore generally found, that methane, that is produced by CO_2 reduction contains less (is more depleted in) ^{13}C than methane, that is produced by acetate fermentation. On the other hand, methane, this is produced from acetate, is usually far more depleted in D than methane, that is produced from H_2/CO_2. This is due mainly to the fact, that all four hydrogen atoms are derived from water in methane, that is produced by CO_2 reduction, whereas three of the four hydrogen atoms are derived from organic matter (acetate) in methane, that is produced by acetate fermentation (Whiticar et al. 1986). A classification diagram for methane, based on data from a number of lake and marine sediments is shown in figure 1.3.

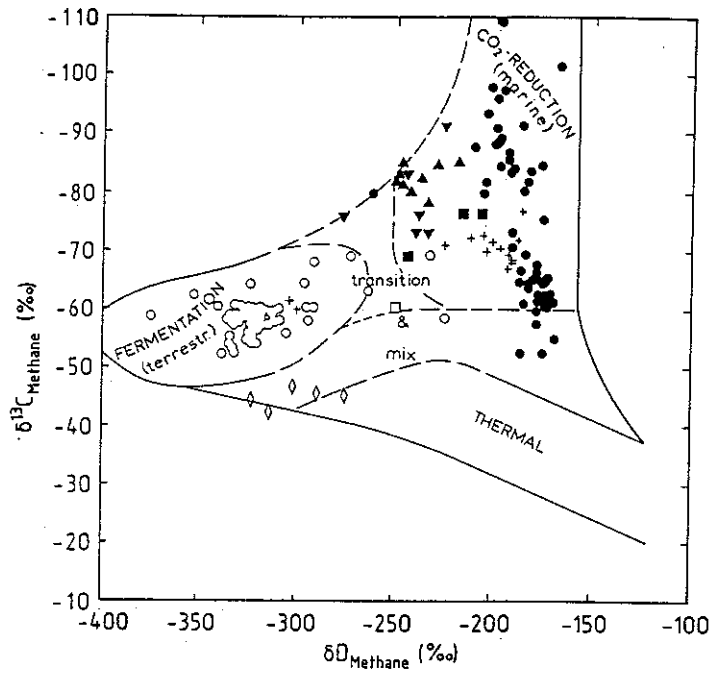

Figure 1.3. Natural gas genetic classification diagram using $\delta^{13}C$ and δD in methane. From (Whiticar et al. 1986).

Thermogenic methane usually have $\delta^{13}C$ values in the -20 to -40 $^{o}/_{oo}$ range, whereas $\delta^{13}C$ values in the -40 to -110 $^{o}/_{oo}$ range indicate a bacterial origin of the methane (Whiticar et al. 1986). Isotopically heavy methane ($\delta^{13}C > -40\ ^{o}/_{oo}$) might however also be the result of methane oxidation (Coleman et al. 1981; Whiticar & Faber, 1986; Alperin & Reeburgh, 1988) or of reservoir effects, when a significant part of the TIC (Total Inorganic Carbon) pool is turned over to methane (Whiticar et al. 1986). Methane produced by acetate fermentation and by CO_2 reduction differ mainly on the δD value, with δD values $< -250\ ^{o}/_{oo}$ being typical for acetate fermentation (Whiticar et al. 1986).

A more recent classification diagram, based on deuterium and used for methane in Canadian aquifers by (Aravena et al. 1995), is shown in figure 1.4. This diagram will be used in section 4.5 to estimate the relative contribution of CO_2 reduction and acetate fermentation to methane production in the Rømø aquifer.

Most studies have shown acetate fermentation to be the primary pathway of methane production in lake sediments (e.g. Kuivila et al. 1988), whereas CO_2 reduction dominates in marine sediments (e.g. Crill & Martens, 1986). All previous studies of bacterial methane from aquifers, that I know of, have showed an isotopic composition of methane, that indicates CO_2 reduction to be the main pathway of methane production in aquifers (Coleman et al. 1988; Grossman et al. 1989; Barker

& Fritz, 1981; Aravena et al. 1995).

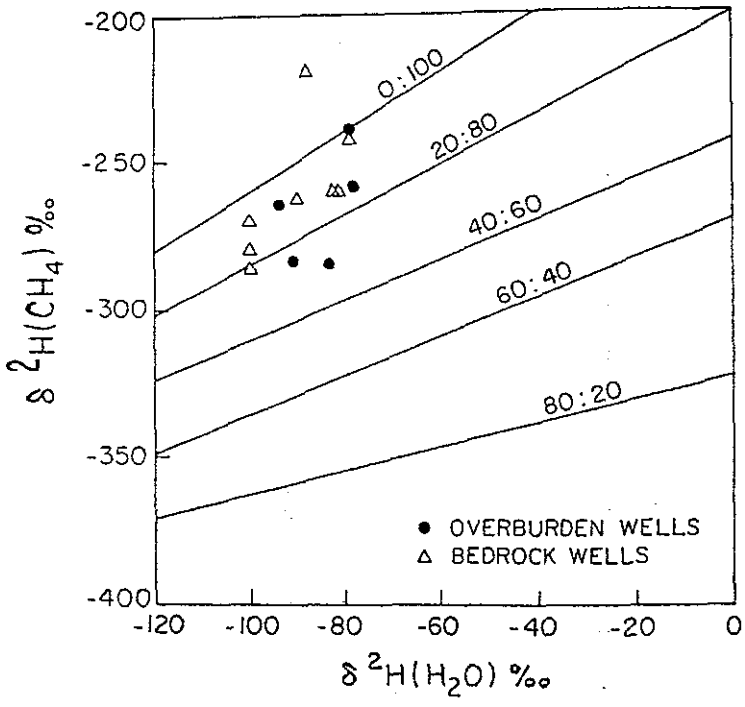

Figure 1.4. Classification diagram for bacterial methane. The line 0:100 indicate 100 % CO_2 reduction, the line 20:80 indicate 20 % acetate fermentation and 80 % CO_2 reduction etc. From (Aravena et al. 1995).

Despite the large number of studies concerning bacterial methane formation in sediments, little is still known about what factors influence the relative importance of the different pathways. Factors, that have been proposed to be important, are the temperature and the age of organic matter (Schoell, 1988). High temperatures and young organic matter are associated with acetate fermentation, whereas low temperatures and old organic matter are associated with CO_2 reduction. (Hoehler, 1998) suggested, that some of the difference in methanogenic pathways between marine and lake sediments might be related to the different pH values and ionic strengths found in these environments.

Besides acetate and H_2, methanogenic bacteria are also able to utilize a few other substrates like formate, methanol, methane thiol, dimethylsulfide and methylated amines (Oremland et al. 1982; Oremland & Polcin, 1982; King, 1984; Oremland, 1988). The latter four substrates are often called "noncompetitive", because they cannot be utilized by sulfate reducing bacteria. Noncompetitive substrates might be responsible for the occurrence of some methane production in sulfate reducing sediments, but have, to my knowledge, only been reported to be significant for the total production of methane in salt marsh sediments (Oremland et al. 1982; Oremland & Polcin, 1982).

Their possible role in the production of methane in aquifers was therefore not investigated in this study.

1.3 Anaerobic methane oxidation

Whereas aerobic methane oxidation is a well understood process, microbiologists have not yet been able to determine, exactly what microorganisms are responsible for anaerobic methane oxidation. It was therefore for many years a relatively controversial subject, despite the large amount of evidence for occurrence of the process, that is provided by several field studies. Anaerobic methane oxidation has been reported to occur both in marine sediments (e.g. Reeburgh, 1980; Devol & Ahmed, 1981; Iversen & Blackburn, 1981; Devol, 1983; Iversen & Jørgensen, 1985; Frind et al. 1990; Blair & Robert, 1995; Hoehler et al. 1994; Niewöhner et al. 1998) and in anoxic water column (e.g. Reeburgh, 1976; Iversen et al. 1987). In both cases, sulfate is supposed to be the main electron acceptor involved in the oxidation of methane as evidenced by concomitant peaks in the rates of methane oxidation and sulfate reduction determined by radiotracers in marine sediments (e.g. Iversen & Blackburn, 1981; Devol, 1983; Iversen & Jørgensen, 1985; Hoehler et al. 1994).

Direct measurements of methane oxidation rates with the use of radiotracers have not been carried out in aquifers prior to this study. In situ rates of methane oxidation in aquifers have however been determined with a natural gradient method by (Smith et al. 1991), who injected methane in shallow aquifers containing oxygen or nitrate. Anaerobic methane oxidation have not been documented in aquifers, but in a recent study of deep aquifers in Texas, (Zhang et al. 1998) reported the finding of methane enriched in ^{13}C, which might indicate that bacterial methane oxidation has occurred (Coleman et al. 1981; Whiticar & Faber, 1986; Alperin & Reeburgh, 1988). (Zhang et al. 1998) found a positive correlation between the ^{13}C content in methane and the concentration of sulfate in the water, which might indicate, that sulfate was the electron acceptor for methane oxidation in the aquifers. It should be noted though, that the concentrations of methane were extremely low in these samples (a few μM), so even if the process occurs, it does not need to be of any quantitative importance.

A possible mechanism for anaerobic methane oxidation was proposed by (Hoehler et al. 1994), who suggested that methane oxidation was carried out by methanogenic bacteria by a reversal of the CO_2 reduction pathway, and that the hydrogen produced by this process was utilized by sulfate reducers. If this theory is correct, methane could be oxidized by a similar pathway with Fe(III) or Mn(IV) as the electron acceptor. All that is needed is a bacteria, that can utilize H_2 and keep its concentration down. Methane oxidation with these electron acceptors has however, at least to my knowledge, not been documented yet.

1.4 In situ measurements of redox rates by the use of radiotracers

The breakthrough in this field started, when (Sorokin, 1962) used ^{35}S for the determination of sulfate reduction rates. (Jørgensen & Fenchel, 1974) developed the method further to an almost in situ method, where the tracer was injected directly into undisturbed sediment cores. Subsequently, this technique has been used for measuring sulfate reduction rates in a number of marine, estuarine, marsh and lake sediments (e.g. Matsumoto & Hanaki, 1990; Phelps & Zeikus, 1985; Iversen & Jørgensen, 1985; Kuivila et al. 1988; Iversen & Blackburn, 1981; Devol, 1983; Hoehler et al. 1994), and more recently also in aquifers by (Jakobsen & Postma, 1994; Jakobsen, 1995). In a quite similar way, the injection of ^{14}C labelled acetate, bicarbonate and methane into undisturbed sediment cores has been used by a number of investigators to estimate in situ rates of methane production from H_2/CO_2 and from acetate (e.g. Crill & Martens, 1986; Hoehler et al. 1994; Harvey et al. 1989; Lansdown et al. 1992; Matsumoto & Hanaki, 1990; Phelps & Zeikus, 1985) and in situ rates of methane oxidation (e.g. Iversen & Blackburn, 1981; Devol, 1983; Iversen & Jørgensen, 1985; Hoehler et al. 1994) in various sediments.

It is generally accepted, that it is almost exclusively the methyl (CH_3) group of acetate, that is reduced to CH_4 by methanogenic bacteria, whereas the carboxyl (COO^-) group is oxidized to CO_2 (Vogels et al. 1988). For this reason, measurements of methane production rates from acetate have been carried out with either 2-^{14}C-acetate, where the methyl carbon is labelled, or with U-^{14}C-acetate, where both carbon atoms are labelled. Oxidation of acetate by other than methanogenic bacteria always results in the formation of two molecules of CO_2. The ratio between formed $^{14}CO_2$ and $^{14}CH_4$ has therefore been used by many authors as an indicator of how large a fraction of the acetate pool is utilized by methanogenic bacteria and how large a fraction by other bacteria (e.g. Sansone & Martens, 1982).

There has been a long dispute about the reliability of rates determined from the turnover of ^{14}C-labelled acetate in marine sediments. Many authors have reported that turnover rates of ^{14}C labelled acetate are much higher than estimates derived from other methods like sulfate reduction rates, ammonium production or known primary productivity. This discrepancy has generally been attributed to overestimation of the biologically available acetate pool due to the existence of a dissolved but biounavailable pool of acetate, that apparently does not exchange rapidly with the injected tracer (e.g. Christensen & Blackburn, 1982; Shaw et al. 1984; Parkes et al. 1984; Novelli et al. 1988; Michelson et al. 1989; Gibson et al. 1989).

In a recent study, (Graaf et al. 1996) demonstrated, that rapid isotopic exchange of the carboxyl group of acetate occurred in a methanogenic lake sediment, presumably because of reversibility of most of the steps involved in the acetate metabolism by methanogenic bacteria. This finding introduces the possibility, that previous studies of acetate turnover in methanogenic sediments,

that have used U-^{14}C-acetate as the tracer, might have seriously overestimated the rate of acetate oxidation as well as the fraction of acetate, that was consumed by other than methanogenic bacteria. It should be noted though, that "too fast" turnover of U-^{14}C-acetate has also been reported in sulfate reducing sediments, and (Graaf et al. 1996) found no evidence for isotope exchange in acetate, when sulfate was the electron acceptor. In this study, 2-^{14}C-acetate was used, which eliminates the problem of isotope exchange of the carboxyl carbon, since only the methyl carbon is labelled. No attempts were made to quantify the size of a possible biologically unavailable pool of dissolved acetate, but as will be shown in the following sections, the acetate turnover rates measured in the Rømø aquifer are generally not suspiciously high compared to other estimates of organic matter decomposition in the aquifer, so this is probably not a serious problem. Perhaps because a sandy aquifer is physically and chemically different from marine sediments in many ways: lower ionic strange of the water, lower adsorption capacity of the sediment due to a much smaller content of clay and organic matter etc.

Most previous measurements of methane oxidation rates with ^{14}C labelled methane also seems to have been hampered by a potentially very serious problem. In a recent study (Harder, 1997) found that the bacterially produced $^{14}CH_4$, that have been used in many previous studies of methane oxidation rates, contains ^{14}CO in amounts of up to ½-1 % of the $^{14}CH_4$. According to (Harder, 1997), CO is readily oxidized to CO_2 by bacteria (e.g. sulfate reducing), that are able to carry out carbonmonooxide-dehydrogenase, and such bacteria are widespread in marine habitats. Since the CO pool in sediments is several orders of magnitude smaller than the CH_4 pool (nmol/l rather than mmol/l), rapid turnover of unintentionally injected ^{14}CO might be responsible for a large fraction of the $^{14}CO_2$ formed in many previous studies of methane oxidation. According to (Harder, 1997) only (Iversen & Jørgensen, 1985) achieved a high enough turnover of the injected CH_4 tracer in their experiments to exclude the possibility, that formed $^{14}CO_2$ was derived solely from contaminant ^{14}CO.

It is uncertain though, to how large an extent the results from previous studies of methane oxidation rates have to be discarded for this reason. The measured rates have usually been supported by concentration profiles and they have been strongly depth dependent despite the likely (although not always documented) presence of sulfate reducing bacteria at all depths. Finally time series have shown linear rates, which indicate, that only one compound in being consumed and that its concentration is not seriously depleted during the incubation. All in all it seems like further studies of CO cycling and methane oxidation in sediments are necessary, before all previous studies of methane oxidation rates using $^{14}CH_4$ are discarded. In this study $^{14}CH_4$ was purified from ^{14}CO by treatment with Hopcalite, as recommended by (Harder, 1997), to avoid the problem.

2. Methods

Many of the methods used are more or less standard methods and will only be described briefly. Others have been developed or implemented as a part of this investigation and will be described and discussed in more detail.

2.1 Collection of water samples

Most of the water samples were taken from driven wells. A filter tip with a 6 cm long stainless steel 50 µm screen was driven down with 1" steel pipe using a pneumatic gasoline driven Pioneer hammer. The filter tips had check valves so that samples could be taken by gas displacement using nitrogen. In May 1996, the samples from 1-5 m.b.s. were taken from a driven well with a stainless steel 50 µm screen, only 1 cm long. The screen was connected directly to a 1/8" stainless steel tubing running inside the driving pipe to the surface. This enabled water to be pumped in small amounts (< 100 ml) from very distinct depths of the aquifer, using a peristaltic pump. In this way an exceptionally low distance between sampling points (2-5 cm) was obtained.

Water for measurements of H_2 concentrations cannot be taken from driven wells, because hammering of the steel pipes leads to production of H_2 (Jakobsen, 1995; Bjerg et al. 1997). For this reason, 16 mm outer diameter PVC tubes, equipped with a 20 µm Nylon screen, were installed in shallow wells, drilled with hand-tools, using plastic casing and a stainless steel bailer. It was found by (Jakobsen, 1995; Bjerg et al. 1997), that this method minimizes the risk of introducing metal to the magazine, that might produce hydrogen through contact with water. It was found by (Bjerg et al. 1997), that a 1-2 month equilibration time is needed after installing such wells, before H_2 concentrations are back to natural levels. For this reason the PVC wells were all installed in December 1996, but left until March 1997, before water was pumped for H_2 analysis.

In July 1997 all water samples were taken from the installed PVC wells, using 8 mm outer diameter teflon tubing and a peristaltic pump. Apart from convenience, this method had the advantage of enabling the determination of H_2 concentrations at the exact same spots in the aquifer, where all other components were determined. Except for some problems with insufficient flushing of the PVC wells prior to sampling for formate and acetate, there were no general differences comparing 1996 to 1997 data, that could be attributed to the different ways of sampling.

All water samples, except those only used for analysis of CH_4, were filtrated in the field, most of them using a 0.2 µm syringe tip filter. Samples for analysis of anions (Cl^-, SO_4^{2-} and NO_3^-), NH_4^+ and organic acids (formate, acetate and propionate) were taken in 5 ml. polypropylene scintillations vials, frozen at the field site and kept frozen (-18 °C) until analysed. Samples for

measurement of cations (Ca, Mg, Na, K and Mn) were taken in 50 ml plastic vials, acidified by adding 0.5 % concentrated nitric acid and stored at 5 °C until analysed. Samples for analysis of DOC were taken in 25 ml or 50 ml. glass vials, equipped with teflon coated screw caps. The glass vials were rinsed by soaking in 30 % nitric acid for 24 hours and subsequent heating to 550 °C. The DOC samples were also acidified, adding 0.2 % concentrated nitric acid, and stored at 5 °C until analysed.

Two different methods were used for collecting water samples for measurement of CH_4 concentrations.

The standard method, that also allowed determination of TIC in the same sample, was to collect water in a syringe (without contact with the atmosphere) and inject it into an evacuated Venoject blood sample vial, that was frozen (upside down) at the field site and kept frozen until shortly (1 hour at most) before analysed. Freezing was necessary mainly to avoid adsorption of CO_2 to the butyl rubber stoppers in the blood sample vials, when TIC was to be determined in these samples. As much as 50 % of the CO_2 and minor amounts of CH_4 was found to disappear from not frozen samples in just 24 hours. Filtration was necessary only when TIC was to be determined, the purpose being to avoid possible contamination from small pieces of carbonate, that would be dissolved by the acid added before analysis to turn HCO_3^- and CO_3^{2-} into CO_2. It was found that this filtration resulted in only minor losses of CH_4, if it was carried out fast. Normal, not gas tight, syringes were used for collection of CH_4+TIC samples, since tests showed the effect of this to be negligible, as long as the transference of samples was done fast.

The second method used for collecting CH_4 samples was implied only for samples, where the isotopic composition of methane was to be determined along with the methane concentration. A 100 ml serum bottle containing 1 ml of concentrated sulfuric acid for conservation purposes was filled with water coming directly from the 8 mm. teflon tube used to transfer water from the bottom of the wells to the surface. The teflon tube was placed near the bottom of the serum bottle to minimize the waters exposure to atmospheric air. The first water, that entered the serum vials, was nonetheless exposed to atmospheric air for some time. It was therefore replaced by overfilling the serum vial for about 5 seconds. The serum bottle was then closed immediately with a teflon coated butyl rubber stopper.

A comparison between the results obtained by these two methods showed, that the CH_4 concentrations found were usually very similar, when < 0.2 mM of CH_4 was measured. When > 0.2 mM of CH_4 was measured, higher concentrations were always found in the serum vials than in the blood sample vials (data not shown). Measurements of CH_4 concentrations in whole undisturbed sediment cores gave results, that were very similar to those found in the serum vials.

This indicates, that the concentrations determined in blood sample vials are slightly lower than the actual concentrations, when the methane concentration is above 0.2 mM.

The reason for this is probably, that water with > 0.2 mM of methane is supersaturated with dissolved gases at surface pressure. Formation of small air bubbles was often observed when water from methanogenic parts of the aquifer was collected in syringes. It seemed impossible to sample these bubbles in a representative way, and it was therefore carefully avoided to transfer them to the blood sample vials. Since TIC was measured in the same samples, these measurements could also have been affected slightly by the observed bubble formation in the syringes. TIC was however present mainly as HCO_3^- in most of the samples, where CH_4 concentrations were > 0.2 mM, which will have minimized loss of TIC to these bubbles. Moreover, CO_2 is much more soluble in water than the other gases present (CH_4 and N_2), and for this reason alone, loss of CO_2 to such bubbles will be much smaller relatively.

2.2 Water analysis

Field measurements included alkalinity by the Gran titration method and Fe^{2+} by the Ferrozine method, originally described by (Stookey, 1970). O_2 and pH were measured, using electrodes, in a closed flow cell, that prevented contact with the atmosphere. O_2 was never found in concentrations, that were significantly above background levels, and therefore no data are shown for O_2.

In the laboratory, anions were determined by IC (Ion Chromatography) with UV (UltraViolet) and EC (Electrical Conductivity) detection. NH_4^+ was determined by FIA (Flow Injection Analysis) and organic acids by IEC (Ion Exclusion Chromatography), using a Dionex AS-10 column and suppressed EC detection. The AS-10 column is specially developed for analysis of low concentrations of organic acids and a Dionex EC suppressor was used to further enhance the sensitivity of the analysis. The analysis of fermentation products is described more detailed in section 2.5. Cations (Na, K, Ca, Mg and Mn) were determined by AAS (Atomic Absorption Spectroscopy) and DOC (Dissolved Organic Carbon) on a DOHRMAN DC 180 TOC Analyser by UV/Persulfate oxidation and IR (InfraRed) detection.

In general, there was a reasonable electro neutrality in the individual samples, with a deviation between the sum of anions and cations of less than 5 %. Some of the samples did however show deviations as large as 10-15 %, but this could generally be attributed to individual errors in the measurement of alkalinity. The analysis of Na^+ was not completely without problems either. An apparent change in the concentration of Na^+ over 4 months at one of the sampling locations showed a large degree of co-variation with the change in deviation from electro neutrality and was therefore attributed to systematic errors in the determination of Na^+.

CH_4 and TIC were measured by injection of 0.2-1.0 ml of headspace gas to a SRI 8680 GC (Gas Chromatograph), equipped with a FID (Flame Ionization Detector) for analysis of CH_4 and other hydrocarbons and a TCD (Thermal Conductivity Detector) for analysis of all gasses. The original concentration in the sample was calculated from the concentration measured in the headspace, the volume of sample and headspace and the solubility constant of the gas. The SRI 8680 GC is portable, and often these analysis were performed during the field trip, sometimes even in the field, to get a quick overview of the redox conditions in the aquifer.

2.3 Sediment analysis

SOC (Sedimentary Organic Carbon) was measured by a method, where the SOC is split into two fractions. One fraction is ADOC (Acid Desorbable Organic Carbon), the other, NADOC (Non Acid Desorbable Organic Carbon) is the organic carbon in the residue. ADOC was determined on a DOHRMAN DC 180 TOC analyser by UV/Persulfate oxidation and IR detection. NADOC and total carbon was measured by IR detection on a LECO CS-225 Carbon-Sulfur detector. SIC (Sedimentary Inorganic Carbon) was calculated as the difference between total carbon and the two organic fractions.

2.4 Measurement of fermentation product concentrations

The measurement of fermentation product concentrations gives rise to special problems, because these components are present in very small concentrations and are quite unstable. Hydrogen has to be measured in the field, as the very small (nM) concentrations change fast during transport or storage. Hydrogen was measured by the bubble stripping method, that was developed by (Chapelle & McMahon, 1991) with the modifications made by (Jakobsen, 1995). The samples were analysed on a **TRACE**ANALYTICAL RGD2 reduced gas detector.

Measurements of organic acid concentrations are not quite so sensitive, since these components are present in concentrations, that are about 3 orders of magnitudes larger than the concentration of hydrogen. Still it is necessary to take great care to avoid pollution of the samples and changes in the concentrations due to microbial activity. Freezing was found to be the best method to avoid the latter problem. It was found, that the concentration of formate and acetate did not change significantly over a four month period in frozen (-18 °C) samples, even though these had been thawed for a few hours, when the first measurement was made. Samples from the Rømø aquifer always showed increasing concentrations of formate and acetate, if they were not kept frozen, presumably because DOC in the samples was decomposed to acetate and formate. In contrast standards prepared in MilliQ always showed decreasing concentrations of formate and acetate, presumably due to oxidation of these. To minimize this problem, 0.2 % chloroform was added to both standards and samples and they were always analysed within 12 hours after thawing or preparation.

Pollution peaks is another very disturbing problem in the analysis of organic acids, particularly when the concentrations are low. To minimize these problems, all glass equipment used for the analysis was rinsed by soaking in 1 M HCl for 12 hours. Even when this was done, there was sometimes pollution peaks in blinds, that had the same retention time as acetate or more often formate. Polypropylene was found to be a much better material than glass with regard to avoiding pollution of organic acid samples, and for that reason 5 ml polypropylene scintillation vials were used for collecting the samples. No adsorption of organic acids to these vials was observed in experiments, perhaps because the samples were kept frozen. In contrast, the use of polypropylene autosampling vials was not possible due to rapid (< 12 hours) adsorption of organic acids to these vials. Adsorption to filters was not a problem, but many kinds of syringe tip filters could not be used, because they polluted the samples with acetate.

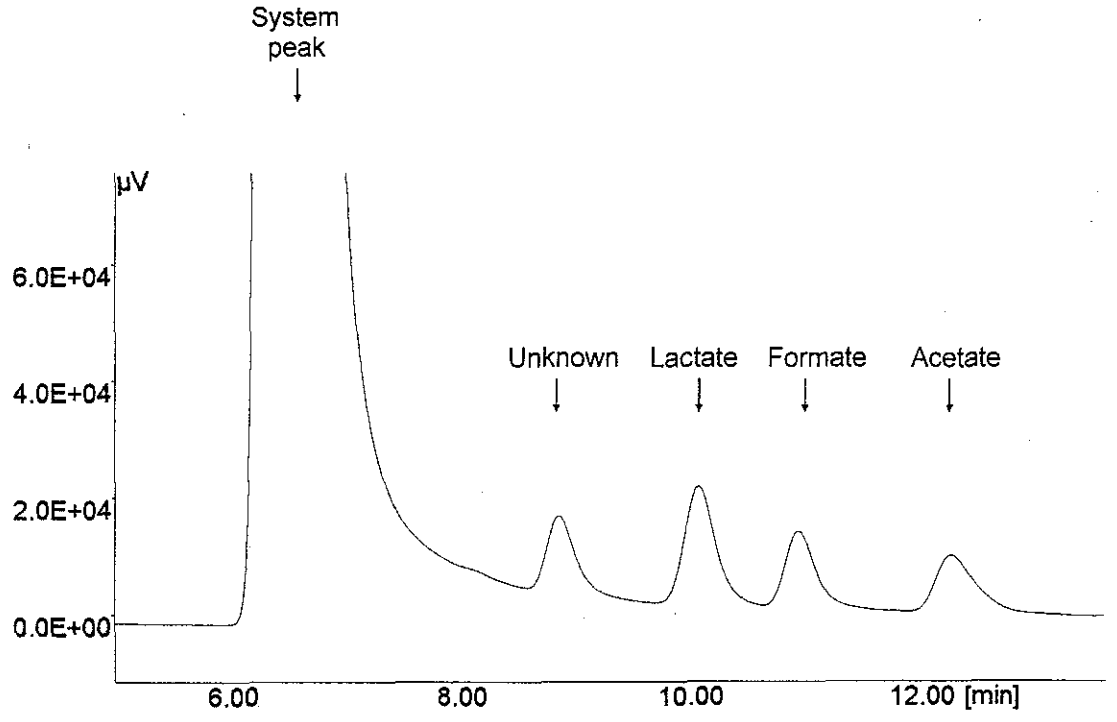

Figure 2.1. Chromatogram showing peaks related to formate and acetate in a sample from the Rømø aquifer.

The detection limit of the method depends strongly on avoiding contaminants and on the natural composition of the sample. If other substances in the sample give large peaks near the retention time of formate and acetate, the formate and acetate peaks might not be detected, if they are very small. In samples without interfering peaks, as little as 0.2 µM formate or acetate could be detected, which is almost one order of magnitude lover than the concentrations measured in the

Rømø aquifer. For this reason it was generally not the theoretical detection limit, but rather the above mentioned problems, that limited the accuracy and precision of this analysis. Propionate was generally not detected in the samples in concentrations above the detection limit, and for that reason it was not attempted to measure higher acids like butyrate. An example of a chromatogram from an analysis of formate and acetate is shown in figure 2.1.

2.5 Determination of in situ rates of redox processes

In situ rates of sulfate reduction, methane production and methane oxidation were measured using 4 different radiotracers: $^{35}SO_4^{2-}$, $H^{14}CO_3^-$, 2-^{14}C-acetate and $^{14}CH_4$.

$^{35}SO_4^{2-}$ was purchased from Amersham in the form of an aqueous $H_2{}^{35}SO_4$ solution. Working standards were prepared by dilution with milli-Q and kept in 2 ml autosampling vials. To avoid any possible risk of changes, the standards were kept frozen until shortly before injection in the cores. An activity of approximately 100 kBq was injected in each sample, which changed the sulfate concentration <<1 %.

$H^{14}CO_3^-$ was purchased from Amersham as an aqueous solution with a pH of 9.2, sealed in gas tight ampoules. Working standards were prepared by dilution with Milli-Q adjusted to a pH of 10.0 with NaOH. The working standards were stored in 10 ml serum bottles, closed with teflon coated butyl rubber stoppers and kept upside down. A layer of Hg was injected in each serum bottle to protect the standard from contact with the butyl rubber stoppers. 100-150 Kbq was injected in each sample, which changed the concentration of TIC << 1 %. The added amount of alkalinity was negligible due to the very small injection volume used (12.5 µl per sample).

2-^{14}C-acetate was purchased from DuPont NEN as an anhydrous crystalline solid (Na-2-^{14}C-acetate) and from Amersham as an aqueous solution of Na-2-^{14}C-acetate. Working standards were prepared by dilution with Milli-Q, stored in 2 ml. autosampling vials, and kept frozen (-18 °C or lower) until shortly before injection into the cores to avoid oxidation of acetate. Possible $^{14}CO_2$, that might have formed by oxidation of 2-^{14}C-acetate prior to freezing, was removed by bubbling the solutions for 20 minutes with N_2. No $^{14}CO_2$ was detected in the standards after this treatment.

The 2-^{14}C-acetate standard from DuPont NEN had a low specific activity of 1-3 mCi/mmol. For that reason only 60 Bq was injected in each sample in October 1996. This resulted in an increase in the acetate concentration of ~1 % at an acetate concentration of 1 µM, assuming that the tracer is equally distributed over the entire core segment. In December 1996, 120 Bq of the same tracer was injected in each core segment, resulting in an increase in the acetate concentration of ~2 %, making the above assumptions. In July 1997, a much more active (59 mCi/mmol) 2-^{14}C-acetate tracer from Amersham was used. 1000 Bq of this tracer was injected in each sample, which

resulted in an increase in the acetate concentration of < 1 %, making the above assumptions.

$^{14}CH_4$ was purchased from Amersham as a gas in evacuated and sealed glass vials. Since only 45 µl of $^{14}CH_4$ was present in each vial, 4-5 ml of N_2 was used to dilute the standard for convenient handling. The diluted $^{14}CH_4$ standard was transferred to a 10 ml evacuated serum vial, using a gas tight syringe. This serum vial contained Hopcalite, that oxidized contaminant ^{14}CO to $^{14}CO_2$, as recommended by (Harder, 1997). The vial was shaken numerous times to facilitate this oxidation. After treatment with Hopcalite, the $^{14}CH_4$ standard was transferred to another serum vial, filled with Hg. In this vial, a small amount of concentrated NaOH was injected, and the vial was shaken to facilitate uptake of $^{14}CO_2$ in the base. The base was then removed from the serum vial and the treatment repeated 5 times to ensure complete removal of $^{14}CO_2$ with minimal loss of $^{14}CH_4$ to the base. Pressure equilibrations during cleaning and injection of $^{14}CH_4$ was made by injecting/removing Hg from the serum vial. After this treatment, no $^{14}CO_2$ or ^{14}CO was detected in the $^{14}CH_4$ standard. Approximately 2000 Bq $^{14}CH_4$ was injected in each sample, which resulted in increases in the CH_4 concentration of << 1 %, except at very low CH_4 concentrations.

It was attempted to minimize the injection of oxygen in the cores by bubbling the various aqueous tracers with N_2 and by using N_2 rather than atmospheric air for dilution of the $^{14}CH_4$ tracer. No attempts were made to assure complete absence of oxygen in the tracers. However, calculations showed, that even at saturation with atmospheric air, the amount of oxygen injected in each sample by the injection of tracer would be used up very fast by inorganic reactions involving e.g. dissolved Fe^{2+}, that was always present in the samples. It is therefore highly unlikely, that the minor amounts of oxygen injected with the tracers could have affected the microbiology in the samples, except very close to the injection lines.

2.5.1 Collection and incubation of samples

Sediment samples for measurements of radiotracer rates were taken in 50/52 mm inner/outer diameter stainless steel tubing, using the system developed by (Starr & Ingleton, 1992). The advantage of this system is, that it is hand operated and that only the sampling tube itself enters the sediment. This leads to minimal risk of disturbing or polluting the sediment collected. To ease sampling, a shallow well with plastic casing was drilled with hand tools for each core to slightly above the depth, where the core was to be taken. At one location (10), each of the long (5 m) cores were taken in one piece, using a 6 m tube. At the other two locations (3 and 8), tube lengths of 3-4 m were used, and each of the long (5 m) cores was taken in two separate tubes. When an upper core had been taken at these locations, a new shallow well was drilled at the exact same spot for collection of a deeper core. The upper samples from these second, deeper cores were taken in sediment, that had been disturbed by the previous coring and by the hand drilling. Still the rates measured in these samples did not deviate significantly from those measured in other samples and

they are therefore thought to be reliable and are included in the data presented in section 4.4.

After collection of a core, it was (generally within one hour) cut into pieces of 25-50 cm, each of them closed with plastic stoppers at the ends and sealed with water and diffusion tight tape. After cutting and sealing, the cores were placed in a hand drilled well with plastic casing for 1-3 hours to bring them back to in situ temperature and to allow eventual settling of the sediment in the core to occur, before injection of tracer was made.

In 1996, injections were made in 40 or 50 cm core pieces (when possible) with 8 cm intervals, the lowest injection being just 4 cm above the bottom of each core piece. Not all of these samples were analysed, which is why larger distances sometimes exists between the sampling points in the rates measured in 1996. Even though the sediment in the ends of the cores could have been influenced by its short contact with atmospheric air, the rates measured at the lowest and highest injection in each core piece did not deviate significantly from the rates measured from other injections in the same cores. Still, to avoid any possible influence from the short contact with atmospheric air, the method was changed slightly. In July 1997 all cores were cut in pieces of 50 cm (when possible) and tracer was injected 7, 18, 29 and 40 cm above the bottom of each core segment. This allowed the upper and lower 3 cm of each core segment to be discarded and 2-3 cm of sediment between each injection point to be used for other analysis.

Tracer was injected through small (1 mm) holes, that were drilled in the steel tube walls immediately before the injection was made. 12.5 - 25.0 ml of tracer was injected as a line through the centre of the core and the holes sealed by water and diffusion tight tape immediately after the injection was finished. After injection, the cores were put back into the hand drilled wells and incubated at the in situ temperature. The range of incubation times used was 12-14 hours for 2-^{14}-C-acetate and 22-25 hours for $H^{14}CO_3^-$, $^{35}SO_4^{2-}$ and $^{14}CH_4$. The incubations were ended by freezing the samples on location and keeping them frozen (-18 °C or lower) until analysed.

2.5.2 Sulfate reduction rates

For determination of sulfate reduction rates, the CRS extraction method, originally described by (Zabina & Volkov, 1978) was applied with a few modifications for separating reduced ^{35}S, formed during the incubations, from unreacted $^{35}SO_4^{2-}$. The frozen samples (7-9 cm of sediment) were placed in pyrex glasses and covered with a 5 % Zn-acetate solution. The purpose of the Zn-acetate solution was to prevent oxidation of sulfides during the subsequent handling of samples. After thawing and homogenisation of the sample, the supernatant was decanted into another pyrex glass and a part of if was filtrated and used for determination of $^{35}SO_4^{2-}$ activities. When decanting was done carefully, no reduced ^{35}S was found in the supernatant. A part of the sediment sample (about

200 g) was then placed in a reaction vessel connected with 1-2 sulfide traps containing 7 or 25 ml of 5 % Zn-acetate and the whole system was made anoxic by bubbling for 20 minutes with N_2, that was purified from oxygen by passing it through a solution of Na-acetate and $FeSO_4$. The separation system is shown in figure 2.2.

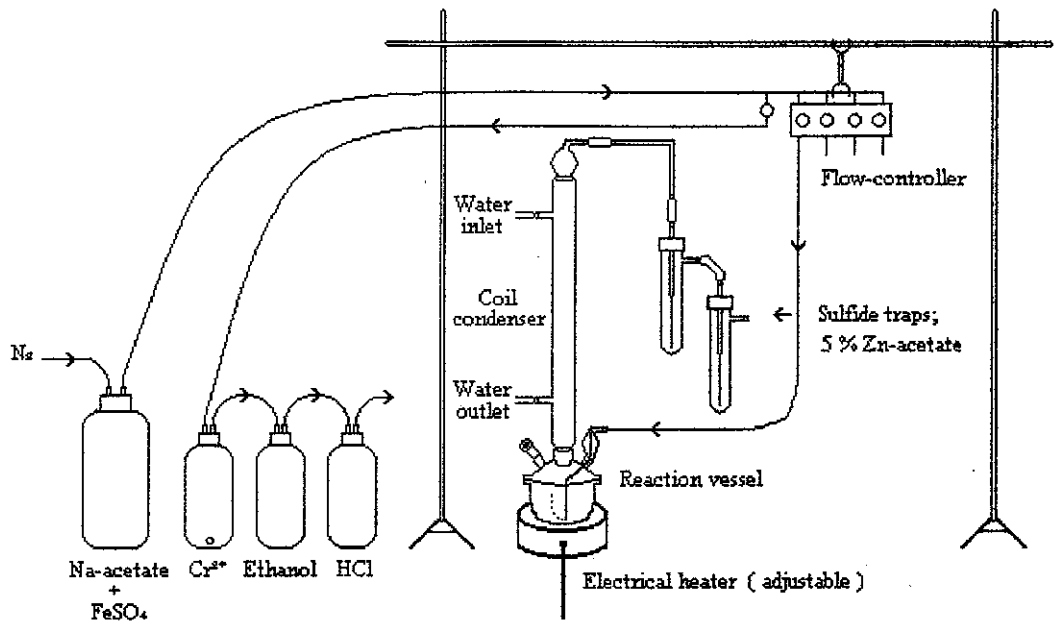

Figure 2.2. The separation system used in the measurement of in situ sulfate reduction rates.

Under continuos bubbling with N_2, the separation was started by adding 20 ml of 8 M HCl and 10 ml of a 1 M Cr(II) solution to each sample. 50 ml of ethanol was added as well, as is improves the efficiency of the reaction. Heating was applied until slight boiling of the water/ethanol mixture occurred and bubbling with N_2 was continued for 90 minutes. Tests showed, that >99 % of the monosulfides and pyrite present in the samples was turned into H_2S and caught in the sulfide traps during these 90 minutes. Tests also showed, that one sulfide trap with 7 ml of 5 % Zn-acetate was sufficient to capture >> 99 % of the sulfide escaping from the reaction vessels, as long as its capacity was not exceeded.

Decanting of the supernatant removed only 70-80 % of the $^{35}SO_4^{2-}$ present, and a coil condenser was therefore used to cool the carrier gas before it entered the sulfide traps. If this was not done, large amounts of $^{35}SO_4^{2-}$ was transported into the sulfide traps as aerosols. Even with the use of a coil condenser, small amounts of ^{35}S accumulated in the sulfide traps when $^{35}SO_4^{2-}$ but no reduced ^{35}S was present in the reaction vessels. This problem was greatly reduced by reducing heating slightly, so only light boiling of the water/ethanol mixture occurred. Therefore only the first few samples analysed had to be corrected for this problem, and the corrections did not exceed 30 % of the measured reduced ^{35}S activity.

The activity of ^{35}S was determined on a Wallac 1414 LSC (Liquid Scintillation Counter) by mixing 7 ml of Zn-acetate/supernatant with 14 ml of Lumagel Safe scintillation liquid (Packard). Quench corrections were made by external standard, using a quench curve prepared by adding different amounts of supernatant to samples with known activities of ^{35}S. Colour in the supernatant was the main quenching factor in these samples. Sulfate concentrations were determined by interpolation from concentrations measured in water, that was centrifuged out of 2-3 cm long core segments between the injections points. The sulfate reduction rates were calculated from:

$$SRR = \frac{(SO_4^{2-}) \cdot a_{red\text{-}sulfur} \cdot \alpha}{a_{sulfate} \cdot t} \quad (1)$$

where SRR is the sulfate reduction rate, (SO_4^{2-}) is the sulfate concentration, $a_{red\text{-}sulfur}$ is the activity of reduced ^{35}S, $a_{sulfate}$ is the activity of ^{35}S in the supernatant, t is the incubation time and α is a fractionation factor.

α is supposed to correct for the discrimination against heavy isotopes in bacterially mediated processes. It cannot be determined accurately for natural environments, and a number of different values can be found in the literature, depending on which strains of sulfate reducers are involved etc. α was set to 1.06 for sulfate reduction in this study.

The activity of ^{35}S found in the supernatant was used for the rate calculation rather than the injected activity. The reason for choosing this method was, that some of the tracer might have been redistributed between the individual samples in each core segment during the incubation and/or subsequent freezing, and it was assumed to be more correct to use the found ^{35}S activity for the calculations. The above rate expression is valid, only when the turnover of injected tracer is low enough to not significantly change the amount of tracer during incubation. Very high turnovers (up to 80 % in some samples) of ^{35}S tracer were observed in samples, where little sulfate (1-20 µM) was present. These high turnovers cannot be assumed to express true in situ rates, particularly not since the actual sulfate concentration is very inaccurately determined at this low concentration level. For that reason, a cut off level of 15 % turnover of ^{35}S tracer was applied, as done by (Jakobsen & Postma, 1994; Jakobsen, 1995). All samples, where this level was exceeded, were discarded from the data. The refinding of ^{35}S tracer was 89.6±12.5 %. Presumably most of the lost tracer was situated in the small segments of the core, that were not analysed for ^{35}S.

2.5.3 Methane production and methane oxidation rates

For measurement of methane production and methane oxidation rates, $^{14}CH_4$ had to be separated from $TI^{14}C$ and 2-^{14}C-acetate. This was done by a two step procedure, where the samples were

initially made alkaline by addition of 50 ml 1 M NaOH to each sample. This resulted in inhibition of microbial processes and in efficient capture of TI^{14}C and 2-^{14}C-acetate in the sample, while $^{14}CH_4$ was either flushed out or held in a headspace. $^{14}CH_4$ was then oxidized to $^{14}CO_2$ and captured in CO_2 traps containing 8-10 ml of either Carbosorb (Packard) or a 1:7 or 1:3 mixture of ethanolamin and 2-methoxyethanol. Ethanolamin and Carbosorb are both amines, that forms strong chemical bindings (carbamates) between 2 molecules of amine and one molecule of CO_2. The sole purpose of 2-methoxyethanol is to facilitate the subsequent mixing of ethanolamin with the scintillation liquid.

Different methods were applied for oxidizing and capturing $^{14}CH_4$ from the samples. Samples for measurement of methane oxidation rates were placed in diffusion tight bags and the methane was allowed to diffuse into the headspace. After an equilibration period of at least 1 week, 1 ml of headspace gas was injected into a SRI 8680 GC and led through a FID detector, where $^{14}CH_4$ was oxidized to $^{14}CO_2$ and caught in a CO_2 trap mounted on the outlet of the FID. One such trap was found to capture >99 % of the $^{14}CO_2$ present. This method was previously used by e.g. (Iversen & Blackburn, 1981) and is indeed very handy, since the concentration of both unlabeled and labelled CH_4 is determined from the same sample in a rapid and simple way.

In principle there is no reason, why this method could not be applied to measurements of methane formation rates as well. But it would require, that a substantial amount of $^{14}CH_4$ had formed during the incubations, since only a minor fraction of this can be injected into the GC. Since only minor amounts of $^{14}CH_4$ had formed in many of the samples analysed in this study, another setup was used for oxidizing and capturing $^{14}CH_4$ when determining methane production rates. The samples (8-9 cm of frozen sediment) were placed in reaction vessels connected with a series of CO_2 traps, and a flow of air was established through the system before base was added and the samples allowed to thaw. 50 ml of 1 M NaOH was added to each sample along with 150 ml of hot tap water to facilitate thawing of the sample. Air was then drawn through the system for 90 minutes during which the reaction vessels were shaken 4-5 times. Tests showed, that >99 % of the formed $^{14}CH_4$ escaped from the samples during these 90 minutes. The separation system is shown in figure 2.3.

The added NaOH effectively retained most of the TI^{14}C and 2-^{14}C-acetate in the samples, but a minor amount of $^{14}CO_2$ escaped from the samples, in which $H^{14}CO_3^-$ had been injected. To separate this from the formed $^{14}CH_4$, the carrier gas was led through a series of CO_2 traps (ethanolamin/2-methoxyethanol), where $^{14}CO_2$ was retained whereas $^{14}CH_4$ passed through. After passage through these CO_2 traps, the carrier gas was led through columns filled with CuO and heated to 800 °C in a tube furnace, a method that was previously used by (Crill & Martens, 1986). The CuO columns were found to oxidize at least 99 % of the $^{14}CH_4$ passing through them into $^{14}CO_2$, as long

as the flow rate was not to high. A column size was chosen (12 mm inner diameter, 25 cm length) that allowed pumping at the maximum speed of the pump without loss of oxidation efficiency.

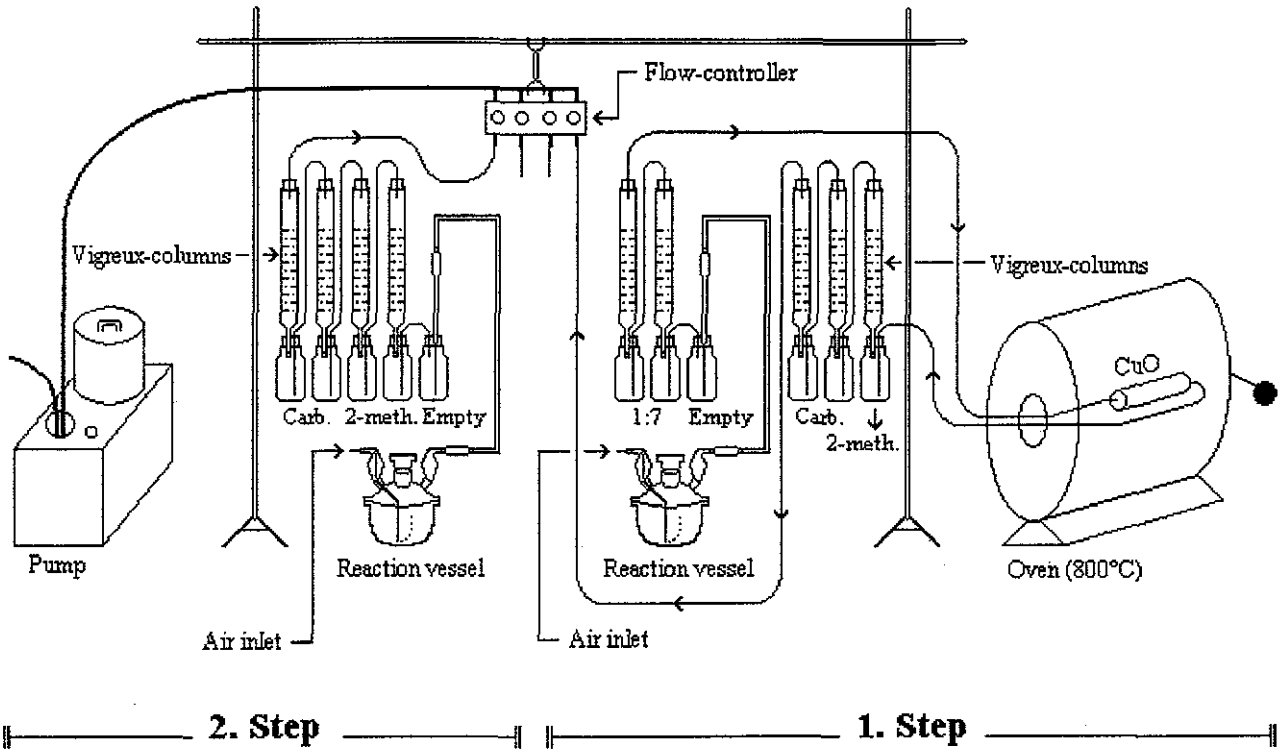

Figure 2.3. The separation system used in the determination of methane production and methane oxidation rates. 2-met. stands for 2-methoxyethanol, 1:7 is a 1:7 mixture of Ethanolamin and 2-methoxyethanol, Carb. stands for Carbosorb.

After passage of the CuO column, the formed $^{14}CO_2$ was captured in another series of CO_2 traps. One CO_2 trap was enough to capture >>99 % of the $^{14}CO_2$ formed by oxidation of $^{14}CH_4$. However, when cleaning traps were used before the oven, some of the ethanolamin and 2-methoxyethanol in these traps evaporated and was oxidized to H_2O and CO_2 in the oven. Due to this, the capacity of a single CO_2 trap was exceeded, and a series of at least two had to be used. A trap containing 10 ml of 2-methoxyethanol was inserted between the oven and the final CO_2 traps to minimize the amount of water entering the CO_2 traps. This was necessary to avoid phase separation, when the CO_2 traps were subsequently mixed with scintillation liquid. The 2-methoxyethanol traps were regularly checked for ^{14}C content, but only very minor amounts were found.

The second step of the separation was identical for all ^{14}C samples. The sample was placed in a reaction vessel connected with a series of CO_2 traps and made acidic by addition of 30 ml 8 N HCl. Air was drawn through the system for 90 minutes during which the reaction vessels were

again shaken 4-5 times. Tests showed, that > 99 % of the $^{14}CO_2$ escaped from the reaction vessels and was caught in the CO_2 traps during this time. The capacity of CO_2 traps was adjusted to the expected carbonate content in the samples, since large amounts of CO_2 forms from dissolving carbonate when acid is added to carbonate containing sediment. Again a vial containing 2-methoxyethanol was inserted before the CO_2 traps to prevent excessive amounts of water vapour from entering these CO_2 traps. Little or no $^{14}CO_2$ was found in these vials with 2-methoxyethanol.

In samples, where 2-^{14}C-acetate was present, a minor amount of this escaped from the reaction vessels in the second acidic step. Tests showed, that approximately 0.2 % of the 2-^{14}C-acetate present escaped during a 90 minutes pumping time. For the great majority of samples, this is very little compared to the amount of 2-^{14}C-acetate, that was turned into TI^{14}C during the incubation. Moreover, the bulk of the 2-^{14}C-acetate, that escaped from the reaction vessels, was caught in the vial with 2-methoxyethanol, that was inserted to remove water vapour. Therefore the amounts of 2-^{14}C-acetate entering the CO_2 traps were very minor. Unreacted 2-^{14}C-acetate was measured in filtrated supernatant, after both separation steps had been carried out.

All ^{14}C samples were counted on a Wallac 1414 (July 1997 data) or Packard TRI-CARB - 1600 TR (1996 data) LSC by mixing 8-10 ml of sample with 10 ml of scintillation liquid. Carbosorb was mixed with Permaflour E, a scintillation cocktail developed by Packard for this purpose. Ethanolamin/2-methoxy-ethanol was mixed with High Safe scintillation liquids like Ultima Gold XR (Packard) or Optiphase HiSafe 3 (Wallac). Supernatant for measuring 2-^{14}C-acetate was mixed with Lumagel Safe (Packard).

Quench corrections were generally made by external standards. At the Packard TRI-CARB - 1600 TR, a quench curve was prepared for the Ethanolamin/2-methoxyethanol system by adding different concentrations of a chemical quenching factor to samples with known activities of $^{14}CO_2$. The determination of 2-^{14}C-acetate on the Packard TRI-CARB - 1600 TR was carried out by internal standards, since colour quench was important in these samples and since a different scintillation liquid was used. The Wallac 1414 has build in quench libraries, and these gave satisfying results, except for measurement of 2-^{14}C-acetate in Lumagel Safe. An adjusted quench curve for this purpose was therefore prepared by adding different concentrations of supernatant to samples with known activity of 2-^{14}C-acetate.

Many of the samples, where $H^{14}CO_3^-$ had been injected, gave such high activities of $^{14}CO_2$ in the CO_2 traps during the second separation step, that $^{14}CO_2$ activities exceeded the value (70000 Bq), where counting is still linear. This problem, a result of the dead time of the counter, was solved, either by dilution of the samples, or by correction of the measured ^{14}C counts, using a prepared correction curve.

The methane production rates from October and December 1996 were measured as part of a M.Sc. thesis by (Nielsen & Holmefjord, 1997). Apparently they had some problems with the determination of 2-^{14}C-acetate in the December 1996 samples by the internal standard procedure mentioned above, since the average refinding of tracer was 165 % in these samples. This is definitely unrealistic, and the measured amounts of 2-^{14}C-acetate were therefore adjusted to give an average refinding of 100 %, before these data were reprinted in this thesis.

As described above, the CH_4 concentrations, that were used to calculate the methane oxidation rates, were determined on the SRI 8680 GC, when headspace gas was injected for oxidation and capturing of $^{14}CH_4$. This means, that the CH_4 concentrations were determined in the samples themselves. In contrast, the TIC and acetate concentrations, that were used to calculate methane production rates, were interpolated from nearby water samples.

For determination of TIC this is not a problem, since TIC is present in millimolar concentrations and therefore practically constant over short vertical distances. Interpolation of the acetate concentrations from nearby water samples is far more problematic. As shown in figure 4.10, there are rather large fluctuations in the measured acetate concentrations over small distances of depth and between repeated sampling at the same location. Besides this, the turnover of acetate is so fast, that large differences in concentration might exist over short vertical and horizontal distances. To eliminate this problem, it was attempted to measure the acetate concentrations in water from the sediment cores themselves. Due to problems with high blinds, this was however given up and the acetate concentrations measured in nearby water samples were used instead. Since fluctuations and peaks in the acetate concentrations measured in nearby water samples do not necessarily reflect the concentrations of acetate in the samples used for rate measurement, a smoothing of the measured concentrations was performed and possibly erratic local peaks in the measured acetate concentrations were neglected.

This procedure was chosen, because these large peaks in the acetate concentrations gave rise to large and apparently unrealistic peaks in the measured acetate turnover rates, if they were used. At location 3 there are rather large deviations between some of the acetate concentrations measured in June, September and October 1996 (figure 4.10). Since these apparent changes might not reflect the concentrations in the sediment cores taken in October 1996, the acetate concentrations at location 3 were calculated from all the values measured in June, September and October 1996. Due to technical problems, acetate concentrations were not determined at location 3 in July 1997, so the acetate concentrations calculated from the June-October 1996 measurements had to be used for calculating the July 1997 rates as well. At location 8 rather large and systematic variations in the acetate concentrations were found from December 1996 to July 1997 and it was chosen to calculate the smoothed acetate concentrations at each time separately. The measured

and smoothed concentrations of acetate are shown in figure 2.4.

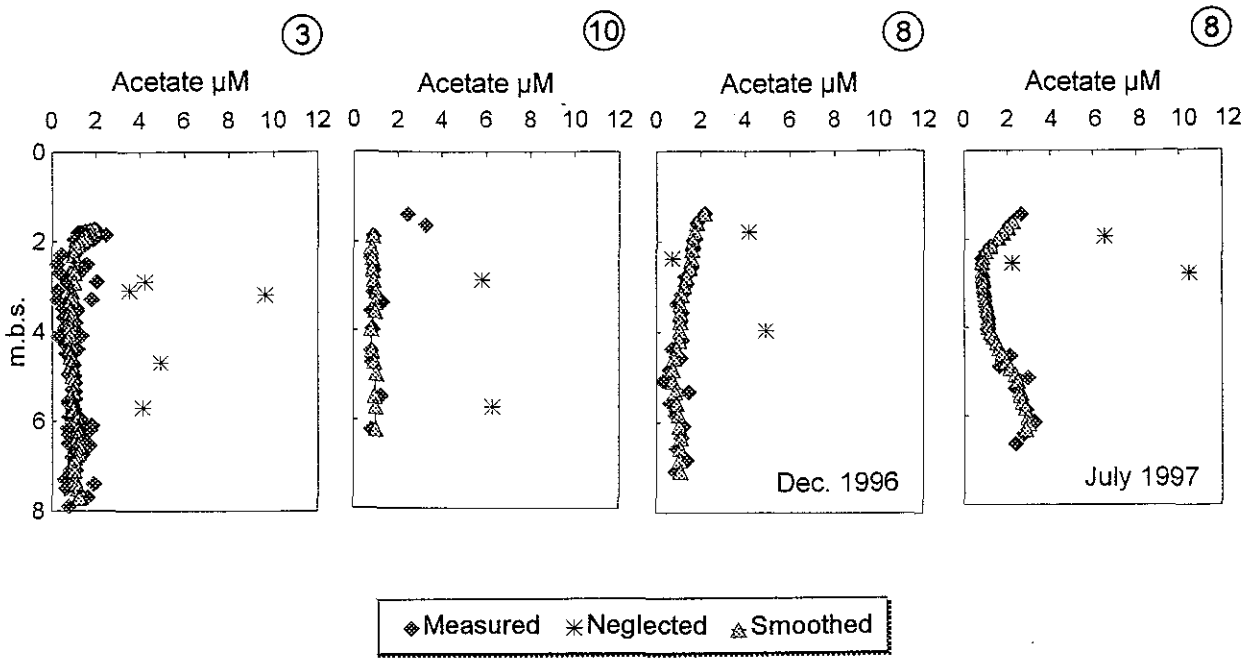

Figure 2.4. Measured and smoothed concentrations of acetate in the Rømø aquifer. The smoothed concentrations were used for calculating acetate turnover rates.

CO_2 reduction and CH_4 oxidation rates were calculated from equations similar to that used for calculating sulfate reduction rates:

(2) $$CRR = \frac{(TIC) \cdot a_{CH4} \cdot \alpha}{a_{TIC} \cdot t}$$

(3) $$MOR = \frac{(CH_4) \cdot a_{TIC} \cdot \alpha}{a_{CH4} \cdot t}$$

where CRR is the CO_2 reduction rate, MOR is the methane oxidation rate, (TIC) and (CH_4) are the concentrations of these compounds, $a_{CH4/TIC}$ is the activities of the various fractions, t is the incubation time and α the fractionation factor. As for sulfate reduction rates, the activity of unreacted tracer was determined from the actual measurement, not from the theoretically injected amount.

α was determined from the difference between the $\delta^{13}C$ values for TIC and CH_4, that were measured in the Rømø aquifer. These measurements showed that the methanogens in the Rømø

aquifer have a fractionation factor between ^{13}C and ^{12}C of 1.04-1.06. It is usually assumed, that the fractionation between ^{14}C and ^{12}C is twice as large as that between ^{13}C and ^{12}C, and α was therefore conservatively set to 1.08 for methane production. Slightly higher fractionation factors are usually reported for CO_2 reduction than for acetate fermentation (Whiticar & Faber, 1986), so perhaps a slightly higher value could have been justified for CO_2 reduction. But given the many other uncertainties involved in these measurements and the large variability in the rates (figure 4.16), an exact determination of α is unimportant for the scope of this study. For CH_4 oxidation much smaller fractionation factors are reported (Whiticar et al. 1986), and these values are highly uncertain. Accordingly no attempt was made to correct the measured CH_4 oxidation rates for isotopic fractionation and α was set to 1.00 for methane oxidation.

The equation used for calculating acetate turnover rates is slightly more complicated, as a large fraction of the injected tracer is turned over during the incubation period. 25-50 % turnover of the acetate tracer was often observed, and in one sample as much as 92.5 % of the tracer was turned over. This means, that it cannot be assumed, that the concentrations of 2-^{14}C-acetate in the samples were constant during the incubations. It is therefore necessary to use a rate expression, where an exponential decrease in the concentration of unreacted tracer over time is assumed, as would be the case, if the rates and unlabeled acetate concentrations were constant over time. Accordingly the acetate turnover rates (ATR) were calculated from:

$$ATR = \frac{\alpha \cdot (acetate)}{t} \cdot \ln \frac{a_{acetate} + a_{TIC} + a_{CH4}}{a_{acetate}} \quad (4)$$

where (acetate) is the concentration of acetate, $a_{CH4/TIC/acetate}$ is the activities measured in the various fractions, α is the fractionation factor (1.08) and t is the incubation time.

The acetate fermentation rates (AFR) and acetate oxidation rates (AOR) were calculated from:

$$AFR = ATR \cdot \frac{a_{CH4}}{a_{CH4} + a_{TIC}} \quad (5)$$

$$AOR = ATR \cdot \frac{a_{TIC}}{a_{CH4} + a_{TIC}} \quad (6)$$

using the acetate turnover rate calculated from (4).

The refinding of tracer was 90.2±8.6 % for 2-^{14}C-acetate and 94.2±7.6 % for $^{14}CO_2$. As for measurement of sulfate reduction rates, most of the lost tracer was presumably located in the small sections of the core, that were not analysed for ^{14}C. For the $^{14}CH_4$ tracer, the average refinding cannot be determined meaningfully, since loss of activity from the standard occurred during field work. Moreover, the amount of $^{14}CH_4$ found in each sample varied highly. Since the refinding of unlabeled methane in the samples was ~100 % based on a comparison between methane concentrations measured in the rate samples and in water samples from the same spot, this can only be because large and highly variable losses of $^{14}CH_4$ tracer occurred during the injections. Alternatively large and highly variable amounts of $^{14}CH_4$ should have been lost between the FID detector and the CO_2 traps mounted on the FID outlet. However, tests showed, that this was not the case. The large and highly variable loss of $^{14}CH_4$ tracer presumably happened, because this tracer was on gas form, whereas the other tracers were dissolved in water. The use of a dissolved CH_4 tracer in future studies is therefore recommended.

In principle it is not a problem for the rate measurement, that the amount of tracer varies highly, as long as the rate is calculated from the actually found amount of tracer, and the injected amount of tracer is low enough to not affect the rates. In this case however, the amount of reaction product formed was so low, that the detection limit of the method was not reached in many samples. In figure 2.5 is shown a comparison between the amounts of $^{14}CH_4$ and $^{14}CO_2$ found in samples, where methane oxidation rates were measured.

At location 3 and 10, most of the samples contained less than 20 DPM $^{14}CO_2$. These low activities give rise to large uncertainty in the LSC process, but worse is, that the correlation between $^{14}CO_2$ and $^{14}CH_4$ is week (R^2= 0.161 at location 3) or completely absent (R^2 = 0.020 at location 10) and that the intercepts of the linear regressions are far from zero. This strongly indicates, that the measurements of $^{14}CO_2$ are subject to systematic errors. At location 8 higher amounts of $^{14}CO_2$ were found in most samples, but the correlation between $^{14}CO_2$ and $^{14}CH_4$ is still not good (R^2 = 0.140) and the regression line at location 8 has an intercept of 22±18 DPM, whereas the similar values are only 5 ± 3 DPM at location 3 and 9 ± 6 DPM at location 10. Apparently the higher $^{14}CO_2$ activities found at location 8 are not completely related to turnover of the injected tracer either.

A closer inspection of the measurements from location 8 reveals, that all the correlation between $^{14}CH_4$ and $^{14}CO_2$ comes from samples above 3 m.b.s. When the data from location 8 are split up at this depth, the resulting correlations are R^2 = 0.381 above 3 m.b.s. and only R^2 = 0.012 below 3 m.b.s. Also the intercept of the regression line is lower for the samples above 3 m.b.s. (16±21 DPM) than for those below 3 m.b.s. (28±15 DPM). In conclusion reliable estimates of methane oxidation rates have only been obtained above 3 m.b.s. at location 8. At larger depths at location

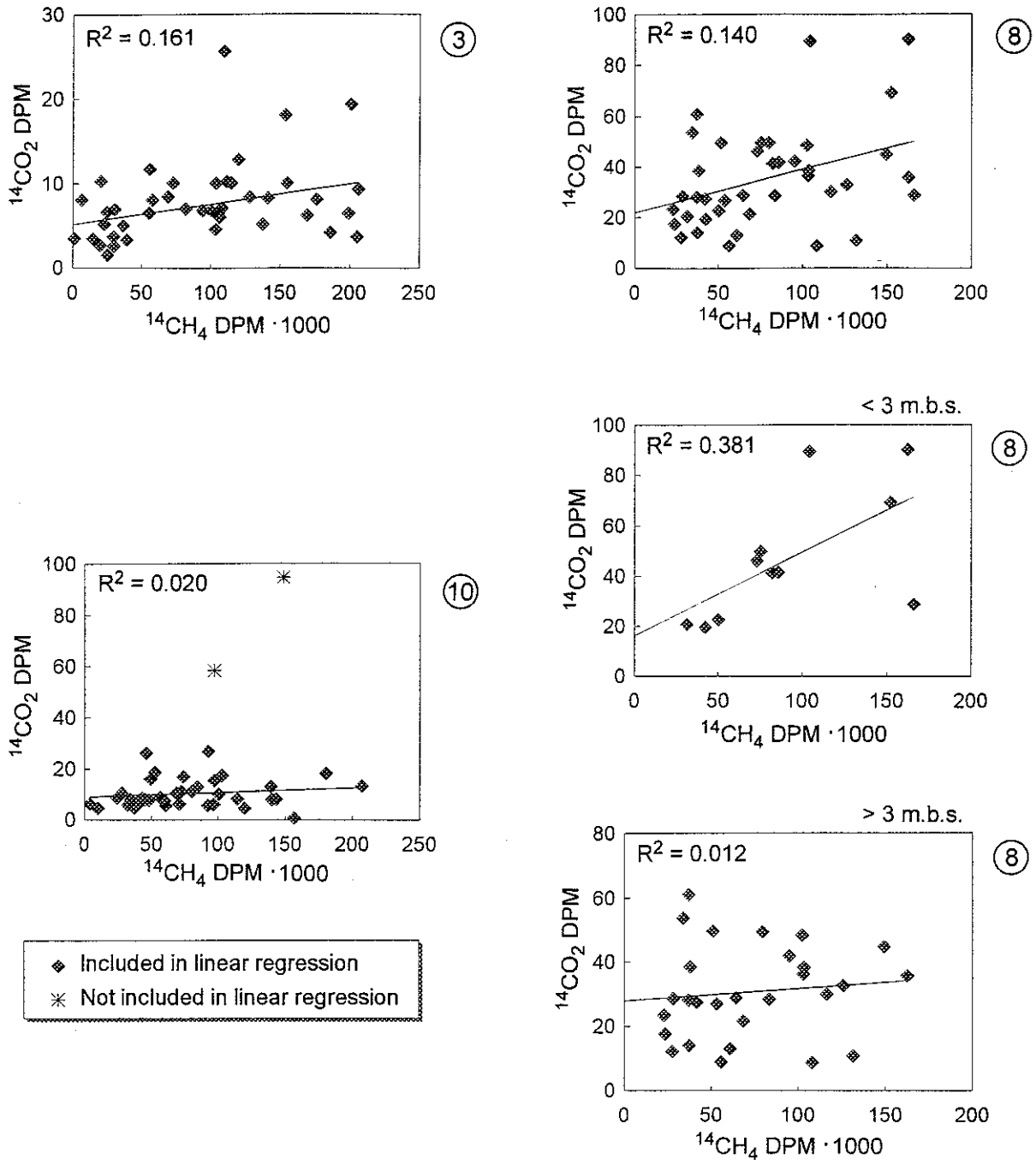

Figure 2.5. Correlation between $^{14}CO_2$ and $^{14}CH_4$ in samples, where methane oxidation rates were measured. Encircled numbers refer to sampling locations. Please note the different scales.

8 and at all depths at location 3 and 10, the measured rates must be considered as maximum estimates only. The actual rates are likely to be much lower, particularly in samples with a low amount of $^{14}CH_4$ injected. To reduce this error, a cutoff level of 50000 DPM $^{14}CH_4$ was used. All samples, where less than 50000 DPM $^{14}CH_4$ was found were neglected in the plot of methane oxidation rates in figure 4.19.

2.6 Time series of in situ rates of redox processes

To check for a possible dependence of the measured rates on the incubation time, a time series experiment was carried out for three of the four tracers used. The sediment for this experiment was collected by repeated coring over a short depth (25 cm) with intermediate withdrawals of the tube to allow new sediment to flow into the space left after coring. In this way the sediment collected in a 2.5 m long tube was taken from the approximate same depth and location in the aquifer, which should reduce the influence of sediment variability on the measured rates. To further reduce the possible influence of sediment variability or sampling procedure, two 25 cm long core segments were used for each incubation time and the position of these within the entire tube length was determined at random. A drawback of this sampling method is, that the sediment is physically disturbed, which is less the case with the normal sampling procedure. As previously explained, rates in physically disturbed sediment from the top of normal cores did not deviate much from the rates in undisturbed sediment, so this difference is not necessarily very important.

However, in the time series experiment, the measured rates were actually different from those, that had been measured 2 weeks earlier, in sediment sampled the normal way at the same spot. This is shown in table 2.1.

Rate	Normal core			Time series		
	mM/yr.	Std. in %	n	mM/yr.	Std. in %	n
Acetate: total	0.328	11.5	3	0.192	17.7	20
Acetate -> CH_4	0.147	10.4	3	0.097	24.6	20
Acetate -> CO_2	0.181	22.0	3	0.096	24.2	20
CO_2 reduction	2.619	46.4	3	0.060	19.3	12
CH_4 oxidation	0.0024		1	0.0013	42.5	20

Table 2.1. Rates measured in the time series experiment and in sediment sampled the normal way 2 weeks earlier at the same spots.

The CO_2 reduction rates measured in the time series are more than 40 times lower than those measured in the normal core. The acetate turnover rates and methane oxidation rates measured in the time series cores (table 2.2) are also lower than those measured in the normal cores. For methane oxidation this is however highly uncertain, since only one sample is available from the normal core.

The reason for the difference in rates between the normal cores and the time series is not known.

It is possible, that the CO_2 reduction rate, and to a smaller extent the acetate turnover rate, simply changed substantially in this part of the aquifer (location 3, 4.80-5.05 m.b.s.) during the two weeks, that separates the measurements. Much lower CO_2 reduction rates were measured at this spot in October 1996 (figure 4.16), so the rates are not necessarily constant over time here. Another possibility is of course, that the physical disturbance of the sediment actually had a large effect on mainly the CO_2 reduction rate. However, such an effect was not seen in physically disturbed sediment from the top of cores sampled the normal way.

The sampling method used in collecting sediment for the time series requires a certain depth below the ground water table, since the sediment must readily flow into the space left after coring. Sulfate was only present at depths < 1 m. below the groundwater table, and it was therefore not attempted to collect sediment for a time series of sulfate reduction rates.

The results of the time series experiment are shown in figure 2.6 and 2.7 The total acetate turnover rate is shown as well as the rates of acetate fermentation and oxidation. Ideally, the calculated rates should be independent of the incubation time and the turnover of tracer should be linearly increasing with incubation time, as long as only little tracer is turned over. Less than 1 % of the injected $H^{14}CO_3$ and $^{14}CH_4$ tracer was turned over, whereas up to 57 % of the injected 2-^{14}C-acetate tracer was turned over in some samples. For this reason linear regression were performed on the turnover of $H^{14}CO_3$ and $^{14}CH_4$ vs. time but not on the turnover of 2-^{14}C-acetate. The rates were calculated as described in section 2.5.3, using the concentrations of TIC and acetate, that had been calculated for an average of the depths, where the sediment was sampled. Concentrations of CH_4 were measured individually in each sample, as also described in section 2.5.3.

The measured rates of acetate turnover seems to be slightly increasing with incubation time. In contrast, there might be a weak tendency, that the measured CO_2 reduction rates are slightly decreasing with incubation time. The turnover of tracer is increasing with incubation time for all three tracers, and the linear regressions of the measured CO_2 reduction and CH_4 oxidation rates indicates, that only a very minor "turnover" of tracer would have been measured in blinds with an incubation time of zero, since the intercepts of the regression lines are very close to zero. This shows, that it is redox processes (or at least other slow processes occurring during the incubations), which result in the finding of turned over tracer, not artefacts in the methods.

To examine the significance of the apparent dependence of the various rates on incubation time, statistical tests (F-tests) were carried out. Such tests require, that the observations: a) are normally distributed b) have means specific for each x-axes point (incubation time) c) have the same variance and d) are independent of each other.

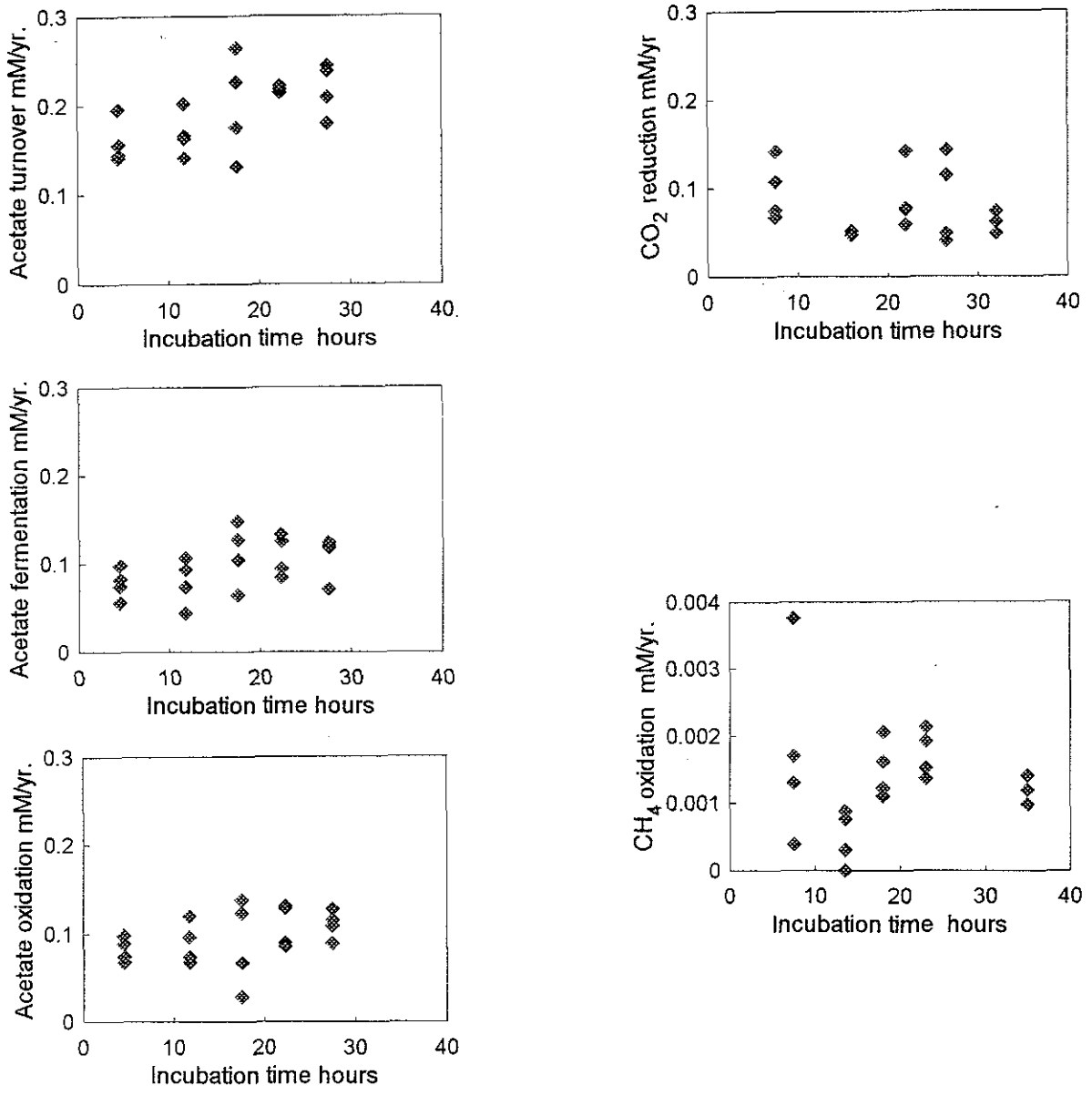

Figure 2.6. Radiotracer rates measured in the time series experiment. Note the different scale for the CH_4 oxidation rates.

There is no strong reason to assume, that the data are not normally distributed. Besides that, minor deviations from normal distribution are not critical for the test. That the measurements have specific means for each incubation time also seems to be a reasonable assumption.

To assume, that the measuring points have the same variance is slightly more problematic. For the acetate turnover rates and CO_2 reduction rates, this seems to be the case. But the CH_4 oxidation rates apparently have a much larger variance at short incubation times than at longer incubation times. This is presumably because the amount of reaction product found in these samples is very low (0-11 DPM). With such low activities in the samples, analytical errors like minor pollution of

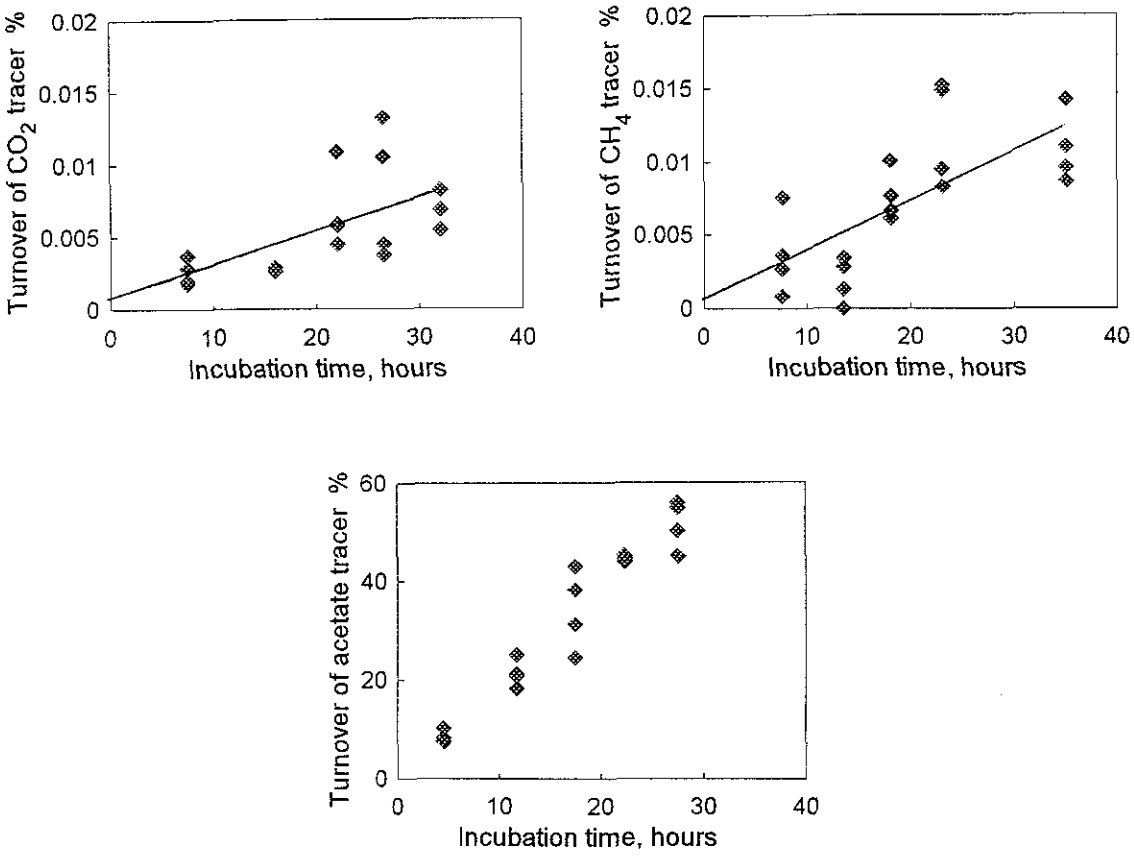

Figure 2.7. Turnover of tracer in the time series experiment. Note the different scale used for the acetate turnover. Solid lines are linear regressions.

samples and counting uncertainty on the LSC contributes significantly to the total variability of the measured rates. Moreover, both of these problems will be more important at low incubation times, where the amount of reaction product is low, leading to a larger uncertainty for rates measured with low incubation times. The calculated standard deviation of the LSC process was 9 % or more for all samples in the CH_4 oxidation time series, and even more at the short incubation times.

In the time series, where $^{14}CO_2$ and 2-^{14}C-acetate tracers were injected, much larger amounts (>200 CPM) of the reaction product was found in each sample. For these tracers, all samples were counted with a standard deviation of < 2 %. This means, that uncertainty in the LSC process does not contribute significantly to the total variability in the measured rates for these tracers. The variability in the acetate turnover and CO_2 reduction rates must be due to variability in the sediment and perhaps to individual errors in the analysis. Since there is no strong reason to assume a dependence between sediment variability (or individual errors) and incubation time, it seems reasonable to assume, that the measured acetate turnover and CO_2 reduction rates have variances,

that are independent of the incubation time.

Assuming that the measured rates are independent of each other is again slightly problematic, since two measuring points with the same incubation time are always located next to each other. To examine the possible influence of this, the measured rates were plotted against the location of the samples in the core, indicated by a number from 1-20. Sample 1-2 are from the lowest 25 cm core segment (the last sediment sampled), etc. For acetate the total turnover rate is shown as well as the rates of acetate oxidation (to CO_2) and fermentation (to CH_4). This plot is shown in figure 2.8.

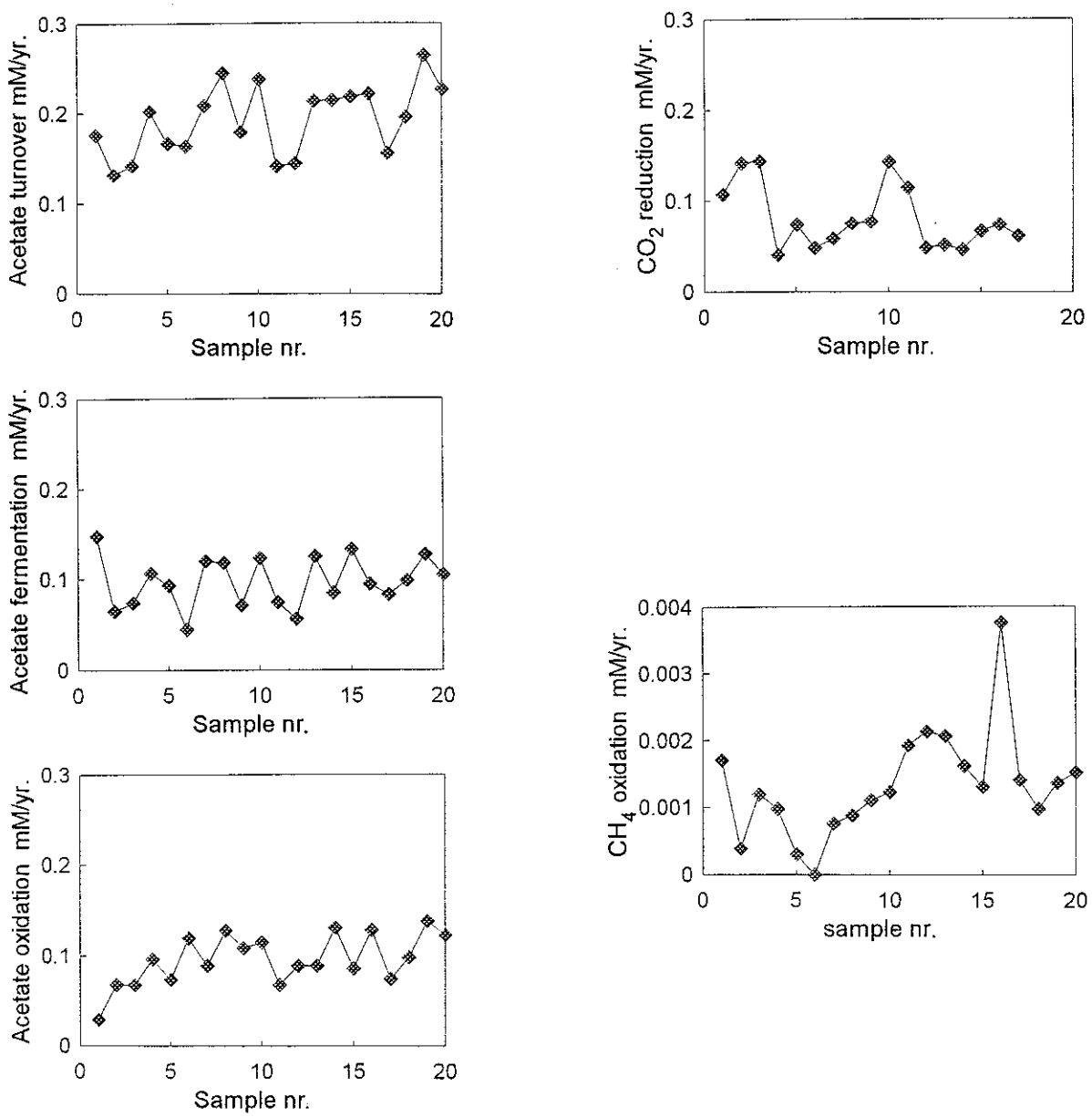

Figure 2.8. Radiotracer rates measured in the time series experiment as function of the position of samples within the sampling tube. Further details are found in the text.

There does not seem to be any systematic variation between the measured acetate turnover rates and the location of samples within the core. On the other hand, there seems to be some systematic variations in the CO_2 reduction rates. There are two distinct parts of the CO_2 reduction core (sample 1-3 and 10-11), where the rates appears to be significantly higher than in the rest of the core.

Run tests were performed to investigate whether it could be assumed, that the measured rates were independent of their location within the sampling tube. This was done by finding the median rate in each set of data and asserting all samples with a higher rate with an "a" and all samples with a lower rate with a "b". The number of runs (e.g. aa/b/aaa/bb etc.) were then counted. For CO_2 reduction the same test was performed, using a rate of 0.1 mM/yr as the dividing point. This was done to test, if the distribution of the 5 samples with rates higher than this could be assumed to be a coincidence. Since this was rejected on a 95 % level, it was assumed, that these five samples actually belonged to a different population and accordingly they were removed from the data and a new test was performed on the remaining 12 samples. Why the rates are higher in these two parts of the CO_2 reduction core is unknown, but it could be due to a few occasions, where the sampling tube was unintentionally driven about 20 cm further than planned. The sediment thus sampled from slightly larger depths might have higher CO_2 reduction rates than the rest. The results of the Run tests are shown in table 2.2.

Rate	Number of observations	Value used to separate data	Number of runs	Critical area (95 %)
Acetate total	20	median rate	10	≤ 6 and ≥ 16
Acetate -> CH4	20	median rate	13	≤ 6 and ≥ 16
Acetate -> CO2	20	median rate	12	≤ 6 and ≥ 16
CO2 reduction	17	median rate	6	≤ 5 and ≥ 14
CO2 reduction	17	0.1 mM/yr	4	≤ 4
CO2 reduction	12 (<0.1mM/yr)	median rate	6	≤ 3 and ≥ 11
CH4 oxidation	20	median rate	5	≤ 6 and ≥ 16

Table 2.2. Run tests for examining the possible dependence of radiotracer rates on position in the sampling tubes in the time series experiment. The hypothesis of independence between measured rates and position in the core is rejected in the critical area.

The hypothesis, that the measured rates are independent of the location within the sampling tube, cannot be rejected on a 95 % level for any of the acetate rates. Moreover, the number of runs are

well within the limits given by the test, indicating that it is very reasonable to assume, that high/low values of the acetate turnover rates are randomly distributed over the tube length. For the CO_2 reduction rates, independence can not be rejected on a 95 % level, when the testing is done using the median rate to divide the data. But it can be rejected on a 80 % level. And if a value of 0.1 mM/yr. is used for dividing the data rather than the median, the hypothesis is rejected on a 95 % level. This indicates, that the five samples with rates higher than 0.1 mM/yr. are not randomly distributed over the tube length. If these five samples are taken out, there is no indication at all, that the remaining 12 samples have rates, that are not randomly distributed over the tube length.

For the CH_4 oxidation rates, the hypothesis of random distribution of high/low rates over the tube length is rejected, mainly because most of the low rates are measured in the bottom of the core (low numbers) and vice versa. The reason for this is unknown, but perhaps the sediment very close to the sampling position had higher CH_4 oxidation rates than the surrounding sediment. It is noteworthy, that the CH_4 oxidation rates forms a relatively smooth curve when plotted against location in the sampling tube, whereas they vary highly when plotted against the incubation time. This indicates, that some of the apparently systematic variations in the measured CH_4 oxidation rates at different incubation times, e.g. the low rates at 13.5 hours, are mainly the result of the different location of samples within the sampling tube.

In conclusion the requirements of the validity of an F-test is met by the acetate turnover rates and CO_2 reduction rates (the 12 samples with rates < 0.1 mM/yr), but not by the methane oxidation rates. However, a visual inspection of the CH_4 oxidation rates does not disclose any systematic dependence on the incubation time, so most likely such a dependence is small or non existent.

To test, whether the acetate turnover and CO_2 reduction rates are independent of the incubation time, it first has to be tested, whether there is a linear dependence between the measured rates and the incubation time. This cannot be rejected on testing levels of 50 % or higher for any of the acetate rates or for the CO_2 reduction rates. Having clarified this, we can now test, again using an F test, whether it can be assumed, that the measured rates are independent of the incubation time (i.e. the slope of the line is 0). This cannot be rejected for the CO_2 reduction rates on testing levels of 50 % or higher. But for the acetate turnover rates, this hypothesis is rejected on testing levels as high as 90 % (acetate oxidation), 95 % (acetate fermentation) and 99.5 % (total acetate turnover). This means, that there is no indication of any dependence between the measured CO_2 reduction rates and incubation times, but a strong indication for such a dependence between the measured acetate turnover rates and incubation time.

The time dependence is not particularly large however. The linear regression of the acetate turnover rates indicate, that 19±7 % higher acetate turnover rates would have been measured, if

an incubation time of 24 hours (as for the other tracers) had been used instead of 12 hours. This value is highly uncertain and it is unknown whether the rates measured at high or low incubation times are most correct. It is also unknown whether the effect of incubation time would be similar on sediment sampled the normal way or from other locations and depths in the aquifer. It was therefore not attempted to correct the measured acetate turnover rates for the apparent time dependence. But it would be relevant to investigate this subject further before more rate measurements are made with the method described here.

Perhaps the production of acetate was stalled by the disturbance of sediment during sampling and the concentration of unlabelled acetate decreased with time at a rate similar to the decrease in the concentration of 2-^{14}C-acetate. If the rate is unaffected by minor changes in the acetate concentration, then the turnover of 2-^{14}C-acetate should be almost linear with time, which indeed seems to be the case (figure 2.7). If the sampling procedure used in the time series experiment disturbed the fermentation of organic matter, it would be expected, that the production of H_2 decreased along with the production of acetate. This might be the explanation for the large decrease in the CO_2 reduction rate compared to sediment, sampled the normal way, two weeks earlier, at the same spot. If this is the case, the time dependency problem does probably not exist in sediment sampled the normal way.

It is also possible however, that the time dependency has other reasons. One such reason could be a large increase in the acetate concentration near the line of injection, where most of the tracer is present shortly after injection. As previously mentioned, the increase in acetate concentration was < 1 %, if it is assumed, that the tracer is distributed equally over the entire sample. However, the tracer is not equally distributed, particularly not shortly after injection. If a small increase in the acetate concentration has little or no effect on the acetate turnover rate, the actual rate will be underestimated due to the artificially increased acetate concentration. This effect will decrease with time because of diffusion and turnover of the injected tracer, and could therefore be responsible for the observed time dependence. If this is the case, the problem can be solved by injecting less tracer and/or by applying a longer incubation time. The latter would however not be practical in the few samples, where a turnover time of acetate down to 5 hours was found (figure 5.4).

3. Geology and hydrogeology of the Rømø aquifer

Rømø is a wadden island situated near the western cost of Jutland. The entire island is formed within the last 2500 years by a continuos deposition of marine sand on the km. wide marine foreland on the western cost of the island. From the marine foreland, sand is wind transported to the eastern part of the island, causing it to grow in height. A map of the island is shown in figure 3.1.

Figure 3.1. Map of Rømø. The investigated site is marked by a ★.

In accordance with the geologic history of the island, the upper 4-5 m of the sediment consists of well sorted aeolian sand, which is underlain by marine sand. Since the sand deposited on the marine foreland is very well sorted compared to other marine sands, the grain size of the marine sand does not differ significantly from the aeolian sand (~0.1-0.2 mm), and investigations using georadar has been unable to determine the depth to the marine sand. However, the presence of marine sand is indicated by a slightly lower degree of sediment sorting and by the presence of thin bands containing more clayey material below 4-5 metres below surface, depending on the topography (Larsen, 1998). A high content of organic and inorganic carbon (shells and shell debris) is also found in these thin bands of more clayey material. The upper unconfined aquifer unit extends down to ~30 m.b.s., where a clay layer is present (Christensen, 1994).

The investigated site is situated approximately 2 km. from the sea and about 500 m. from the groundwater divide (Jakobsen, 1995). The annual infiltration was calculated by (Christensen, 1994) to be 450 mm in 1992 which was a fairly wet year with 820 mm of precipitation (the average is 750 mm). Subtracting slightly from the 450 mm to obtain a more normal precipitation and assuming a porosity of 30 %, the vertical movement of water in the aquifer must be about 1.25 m/yr. The horizontal flow velocity is slightly more uncertain, but is generally around 10 m/yr. in the upper part of the aquifer (Jakobsen, 1995). This low horizontal flow velocity makes the aquifer ideal for detailed geochemical investigations, since the water sampled in a vertical profile has infiltrated over a short distance. A map of the field site is shown in figure 3.2.

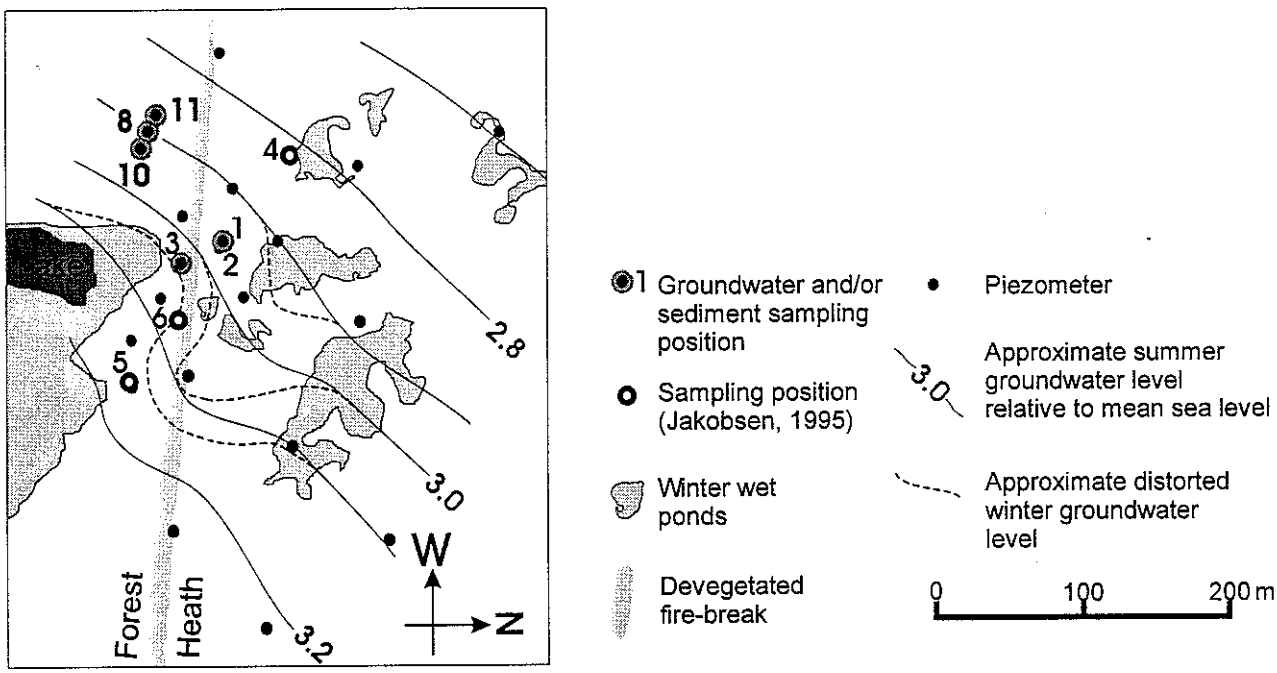

Figure 3.2. Map of the field site including approximate winter and summer groundwater levels and sampling locations. Modified after (Jakobsen 1998) with three new sampling locations (8, 10 and 11) added.

An E-W oriented devegetated fire control belt cuts through the investigated site. North of this belt, an open heath is present, whereas coniferous forest is present in the western and eastern part of the area south of the vegetation free belt. A large winter wet pond, including a small lake, is present in the southern part of the field site and several small winter wet ponds are found on the heath. Expect from the winter wet ponds and the small lake, several small (½-1½ m high) sand dunes dominate the topography of the area. The thickness of the unsaturated zone varies from 0-2½ m., depending on the topography and season, since the groundwater table changes at least ½ metre during the year. Sandy soils in Jutland with heath or coniferous forest vegetation usually shows a well developed podzolization profile (Petersen, 1976). Presumably such a profile will

eventually be formed on Rømø too, but due to the young age of the Island, no clear podzolization profile exists yet.

The general flow direction in the aquifer is SE→NV (figure 3.2). However the large winter wet pond in the southern part of the area causes a seasonal change in the flow direction in parts of the aquifer, most profoundly near location 3, that is situated very close to the winter wet pond. The flow velocity at location 3 is also higher during the winter as indicates to the close lying equi potential lines in the winter situation.

Detailed geochemical studies have been carried out by (Jakobsen & Postma, 1994; Jakobsen, 1995; Larsen, 1998) at location 1-6. However, at the time of this investigation, methane was only found at location 3, and accordingly three new sampling locations (8,10 and 11), all situated along a flow line in the western part of the area (figure 3.2), were chosen for the investigation along with location 3. Location 10 is situated on the top of a 1-1½ m high sand dune, and the distance to the groundwater table is 1-1½ m larger than at the other locations. To enable a better comparison of the results from the various sampling locations, all depths are shown relative to a fixpoint at location 3. The term "x m.b.s." means, that the sample was taken x metres below a level, that corresponds to the terrain at location 3. Since the water table only differed by 9 cm between the sampled locations in July 1997, the corrected depths are also comparable with respect to the groundwater table. The groundwater table is not shown in the figures, because it varies at least ½ metre during the year, but the upper samples shown are generally not more than ½ m. below the groundwater table at the time of sampling, sometimes even much less.

4. Results

4.1 General water chemistry

The general water chemistry in the Rømø aquifer was described by (Jakobsen, 1995), who found large variations between the 5 sampled locations. As there might also be temporal variations, location 3 was resampled for this investigation together with the new locations 8 and 10 (figure 3.2). A cumulative plot of the major cations and anions at the various locations is shown in figure 4.1.

The total solute concentration varies from 3-7 meq/l at the three sampling locations. The general concentration level of Cl^- is ~ 2 mM at all locations. In 1996 elevated Cl^- concentrations of up to 4 mM were found at location 8 from 1.4 to 2.4 m.b.s.. These elevated Cl^- concentrations are probably the result of dry deposition of sea salt during winter storms at location 8, which is situated at the edge of the forest. At location 10, situated just 13 m upstream from location 8, but behind the forest edge, elevated Cl^- concentrations were not found in 1996.

To check for temporal variations in the general water chemistry, location 3 was resampled for all major ions in September 1996, four months after the initial sampling. In July 1997, all locations were resampled for Cl^-. The results are shown in figure 4.2.

The general water chemistry did not change much at location 3 from May to September 1996. The only major ion, that show some general change in concentration, is Na^+. This is somewhat puzzling, since the concentrations of Cl^- and all other major ions remained practically constant. A closer inspection reveals a considerable covariation between the changes in Na^+ concentration and the changes in deviation from electro neutrality from May to September (data not shown). Accordingly, erroneous measurements of Na^+ is the most likely explanation for the apparent change in Na^+ concentration from May to September 1996. The high concentrations of Cl^-, Na^+ and Mg^{2+} in the upper sample from September 1996 are probably a result of evaporation due to a falling groundwater table during the summer, where little or no infiltration occurred.

A large peak exists in the concentrations of K^+ and Mg^{2+} at location 3, at appr. 6 m.b.s. The reason for this is not known, but it could be a result of ion exchange reactions following from a past salt pulse passing through this part of the aquifer. The repeated measurement of Cl^- concentrations in July 1997 revealed no major changes in the deeper parts of the aquifer. But at location 10 a salt peak infiltrated the aquifer during the winter of 1996-1997, raising the concentration of Cl^- from 2 - 3.5 mM above 2 m.b.s. At location 3 and 8, the Cl^- concentration remained relatively constant from 1996 to July 1997 except for a narrow peak at location 8, 2 m.b.s.

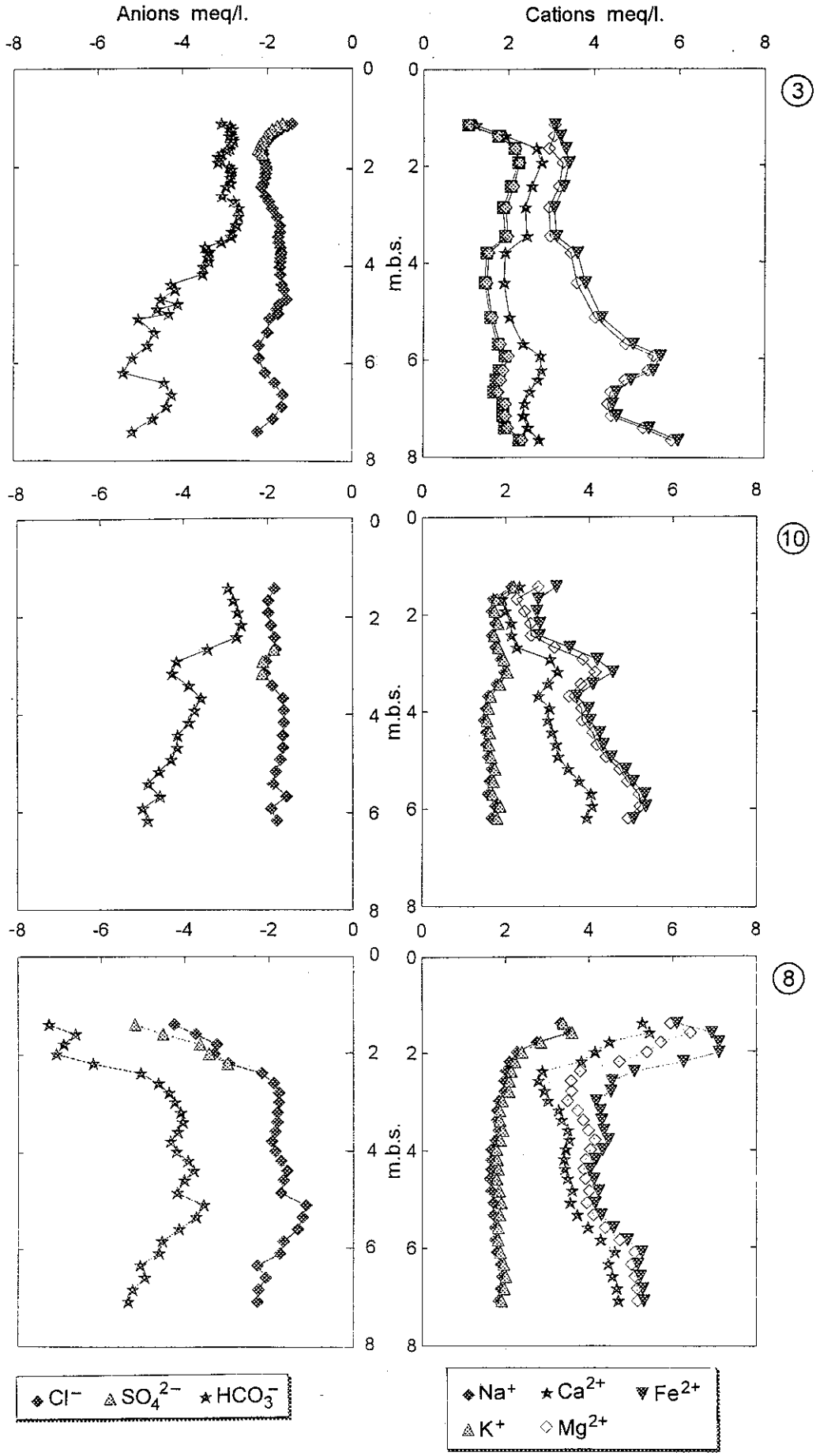

Figure 4.1: Cumulative plots of major cations and anions in the Rømø aquifer. The samples were taken in May 1996 (location 3) and December 1996 (location 10 and 8). Encircled numbers refer to sampling locations.

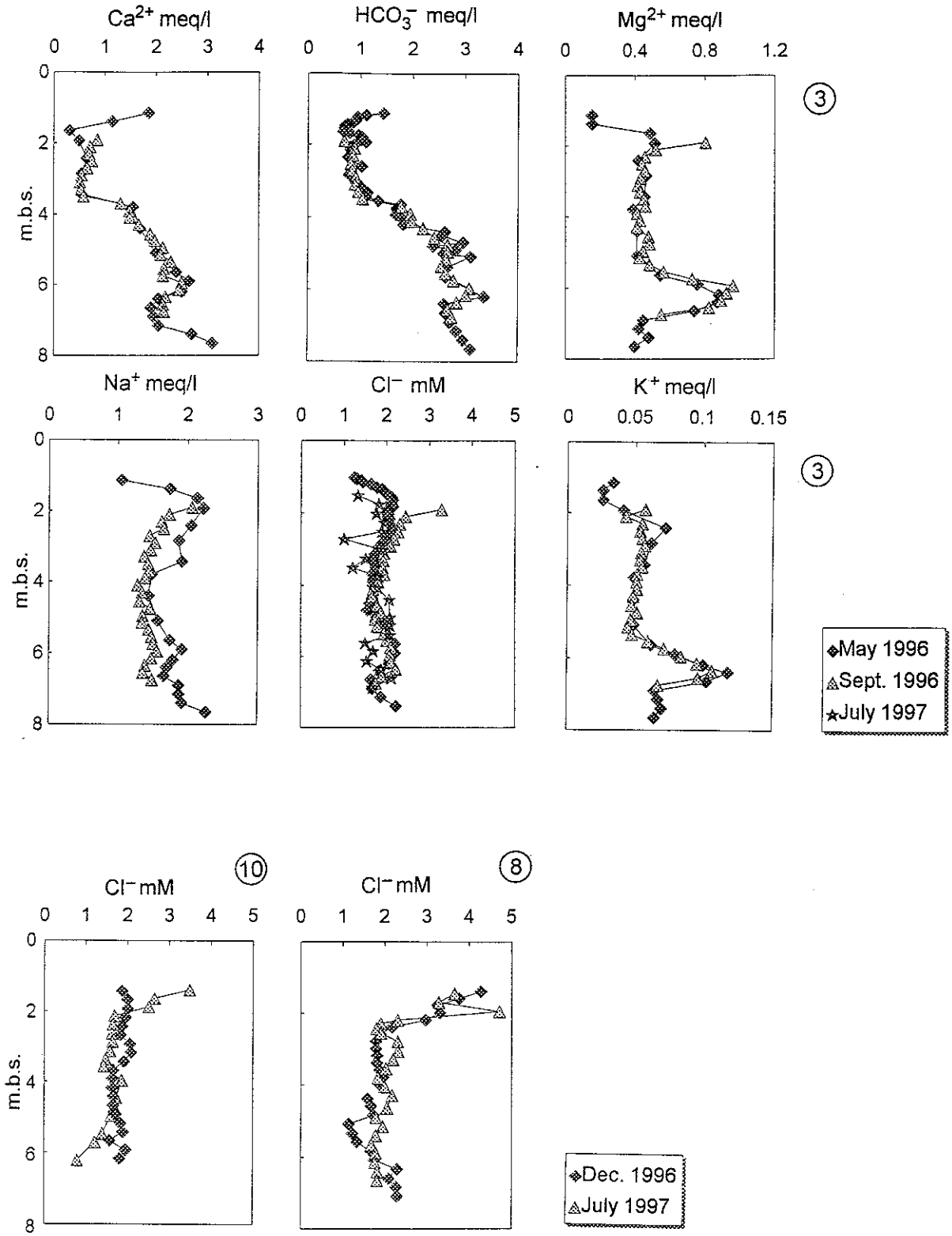

Figure 4.2. Temporal variations in the general water chemistry in the Rømø aquifer. Encircled numbers refer to sampling locations.

The water chemistry shows a clear influence of Ca-carbonate dissolution (figure 4.3). The Ca^{2+} concentration increase sharply at a certain depth at all three locations, and then continues to increase slowly with depth, until equilibrium with calcite is approached in the deeper parts of the aquifer. At location 3 and 10, the increase in Ca^{2+} concentration is accompanied by an increase in pH from 6.2-6.5 to 7.3-7.5 at the same depths where the Ca^{2+} concentration starts to increase. Alkalinity also increases at location 3 and 10 at the same depths as Ca^{2+} and pH, exactly as would be expected, if calcite dissolution occur according to (7).

(7) $$CaCO_3 + CO_2 + H_2O \rightarrow Ca^{2+} + 2HCO_3^-$$

Most of the Ca-carbonates in the Rømø aquifer are shells and shell fragments of mussels. Accordingly, calcite is probably not a realistic representative of the Ca-carbonates, but this is unimportant for the scope of this study. What is important is, that the pH, alkalinity and Ca^{2+} concentration in parts of the aquifer is controlled by dissolution of Ca-carbonates.

At location 8 the concentration of calcium was high (2 meq/l) above 2 m.b.s. in December 1996, then decreased sharply around 2 m.b.s and increased sharply again around 3 m.b.s. This most likely reflects an inhomogeneous distribution of shell fragments in the sediment shortly upstream from location 8. Location 8 is situated downstream a large sand dune, where large amounts of shell fragments might have been wind deposited. The generally much lower alkalinity above 3 m.b.s. at location 8 in July 1997 (figure 4.3) suggests that less Ca-carbonate had been dissolved in the water found here in July 1997. So apparently the dissolution of calcite vary highly along slightly different flow paths in this part of the aquifer. In accordance with the distribution over depth of Ca^{2+}, pH and alkalinity is also higher in the upper part of the aquifer at location 8 than at the other two locations. This, however, is also likely to be the result of Fe(III)-oxide reduction, as will be shown in the next section.

The SI (Saturation Index) for calcite and the alkalinity in July 1997, both shown in figure 4.3, was calculated with PHREEQC (Parkhurst & Appelo, 1997) entering values of pH, TIC, SO_4^{2-} and Fe^{2+} measured in July 1997 and concentrations of all other cations (including Ca^{2+}) measured in September/December 1996. Cl^- concentrations were adjusted to achieve electro neutrality and depths were adjusted to each other by interpolation of the 1996 data. This less than ideal procedure was necessary, because no complete water analysis were available, where all components had been determined at the same time.

Given the large deviation between the alkalinity 1.5-2.7 m.b.s. at location 8 in December 1996 and July 1997, it is reasonable to assume, that $SI_{Calcite}$ also changed substantially here between these

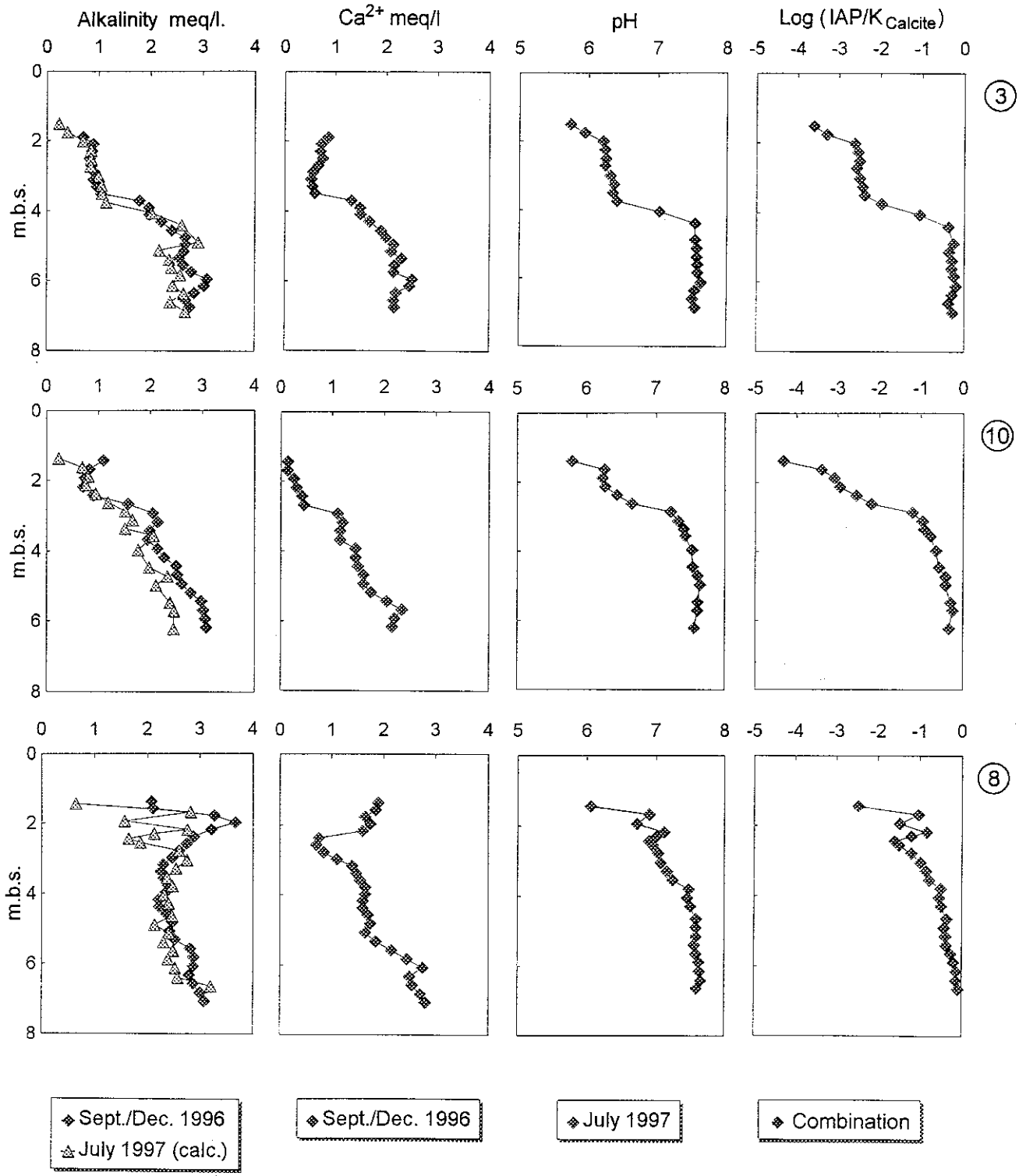

Figure 4.3. Carbonate chemistry at three sampling locations (encircled numbers) in the Rømø aquifer. Details on the calculations are found in the text.

two sampling times. $Si_{Calcite}$ was probably higher than shown in December 1996 and lower than shown in July 1997 in this part of the aquifer. It is even possible, that saturation for calcite was approximated in December 1996. At larger depths and at the other two locations, the alkalinity changed only slightly from 1996 to 1997. This makes it unlikely, that $SI_{Calcite}$ changed substantially here from 1996 to 1997, and the calculated $SI_{Calcite}$ is probably representative for both sampling times.

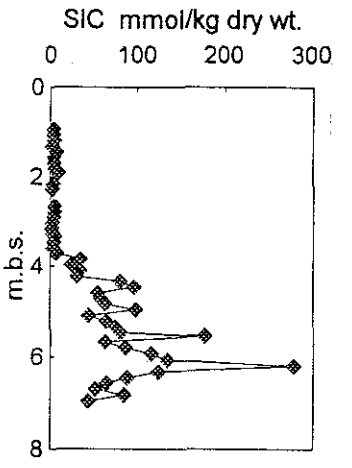

Figure 4.4. SIC content in the sediment at location 3 in the Rømø aquifer.

At location 3 the SIC (Sedimentary Inorganic Carbon) content in the sediment was determined along with the determination of SOC (Sedimentary Organic Carbon). The results are shown in Figure 4.4 and agree well with the measured water chemistry. No or little SIC is present in the sediment at location 3 above the depth (4 m.b.s.), where pH increases along with the Ca^{2+} concentration and alkalinity.

4.2 Redox chemistry

4.2.1 Redox sensitive solutes

The simplest method to determine the redox conditions in sediments is to look at the disappearance of substances being reduced, e.g. O_2, NO_3^- or SO_4^{2-}, and at the appearance of reaction products like CH_4, Fe^{2+}, Mn^{2+} or H_2S. Unfortunately the picture might be disturbed by further reaction of the reaction products. H_2S produced by sulfate reduction might precipitate as FeS or FeS_2, if dissolved Fe^{2+} or reactive Fe(III)-oxides are available. Fe^{2+}, formed by Fe(III)-oxide reduction, might precipitate not only as FeS or FeS_2, but also as siderite ($FeCO_3$). Since the activity of CO_3^{2-} is strongly dependent on pH, the latter reaction is mainly seen at high or neutral pH values. Saturation calculations showed that Siderite is a realistic phase in the Rømø aquifer. The concentration of dissolved Fe^{2+} is also subject to ion exchange reactions with the sediment and to sorption to organic matter. Finally CH_4 might be reoxidized to HCO_3^- or transported upwards as bubbles, if the water gets supersaturated with dissolved gasses. Also, temporal changes in the composition of the infiltrating water, along with horizontal differences in the sediment, will affect the water composition along the different flow lines intersected by a vertical concentration depth profile. This might lead to false conclusions, if concentration gradients alone are used to estimate rates of redox processes.

Yet, while this simple method is far from perfect, it is still a feasible one for getting a rough

overview of the redox processes occurring in an aquifer. Concentrations of O_2 and NO_3^- are always below the detection limit in the Rømø aquifer, even in samples taken just 10-20 cm. below the groundwater table. H_2S concentrations are also generally below the detection limit (1 μM), presumably because there is always enough dissolved Fe^{2+} in the water to cause precipitation of FeS or FeS_2. The most relevant redox sensitive solutes to look at in the Rømø aquifer are therefore: Mn^{2+}, Fe^{2+}, SO_4^{2-} and CH_4. Concentration depth profiles of these solutes are shown in figure 4.5.

Judging from the concentration depth profiles for Mn^{2+}, the reduction of Mn(IV)-oxides is only of any quantitative importance at location 8, where the Mn^{2+} concentration reaches a level corresponding to roughly 10 % of the highest Fe^{2+} concentration. At location 3 and 10, the highest Mn^{2+} concentrations are only 3-4 % of the highest Fe^{2+} concentrations, and Mn(IV)-oxide reduction is not of any quantitative importance. At all three locations, the Mn^{2+} concentration reaches a maximum not more than ½ m. below the groundwater table, indicating Mn(IV)-oxide reduction to be occurring mainly in the uppermost part of the aquifer.

At all three locations, the Fe^{2+} concentration starts to increase not more than 20-30 cm. below the groundwater table, and the highest Fe^{2+} concentrations are found less than 1 m below the groundwater table, but at slightly greater depths than the highest Mn^{2+} concentrations. This indicates, that Fe(III)-oxide reduction is taking place in the upper part of the aquifer at all three sampling locations. Below 2 m.b.s., the concentration of Fe^{2+} is generally constant or decreases with depth and does therefore not indicate ongoing Fe(III)-oxide reduction. There seems to be a positive correlation between the presence of sulfate in the water and the concentration of Fe^{2+}. Firstly, the Fe^{2+} concentration is highest in the upper, sulfate containing, part of the aquifer at all three locations. Moreover, a second peak in the Fe^{2+} concentration was present 3 m.b.s. at location 10 in December 1996, coinciding with low, but significantly above background, concentrations of sulfate. In July 1997 only background sulfate concentrations were found here and the Fe^{2+} concentration was significantly lower.

Judging from the concentration depth profiles of Fe^{2+}, the reduction of Fe(III)-oxides does not occur in the sulfate free parts of the aquifer. It is possible though, that Fe(III)-oxide reduction actually occurs here, but that the resulting Fe^{2+} is removed from solution by precipitation, ion exchange reactions or adsorption to organic matter. To investigate if Fe^{2+} could be removed from solution by precipitation of siderite, the $SI_{Siderite}$ was calculated with PHREEQC (Parkhurst & Appelo, 1997) using the same parameters as for calculating $SI_{Calcite}$. Since the pH, alkalinity and Fe^{2+} concentrations entered were measured in July 1997, the calculated $SI_{Siderite}$ should represent the situation in July 1997. The facts that the concentrations of cations other than Fe^{2+} were measured in 1996 is only of minor importance for the calculation of $SI_{Siderite}$, since their

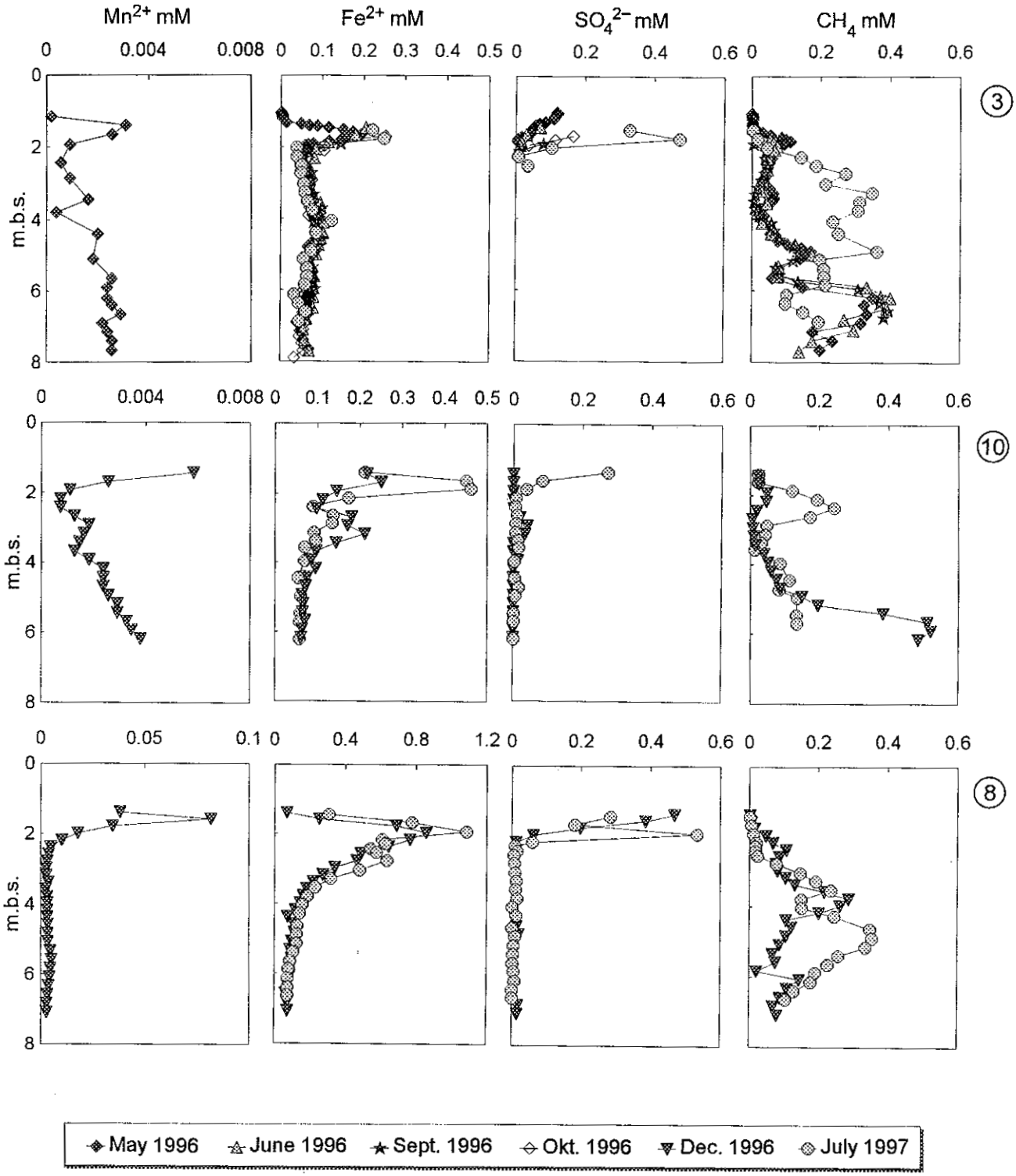

Figure 4.5. Concentrations of redox sensitive solutes in the Rømø aquifer. Note the different scales used for Fe^{2+} and Mn^{2+}. Encircled numbers refer to sampling locations.

concentrations are relatively constant over time and only influence the complexation and activity corrections. The calculated $SI_{Siderite}$ values are shown in figure 4.6.

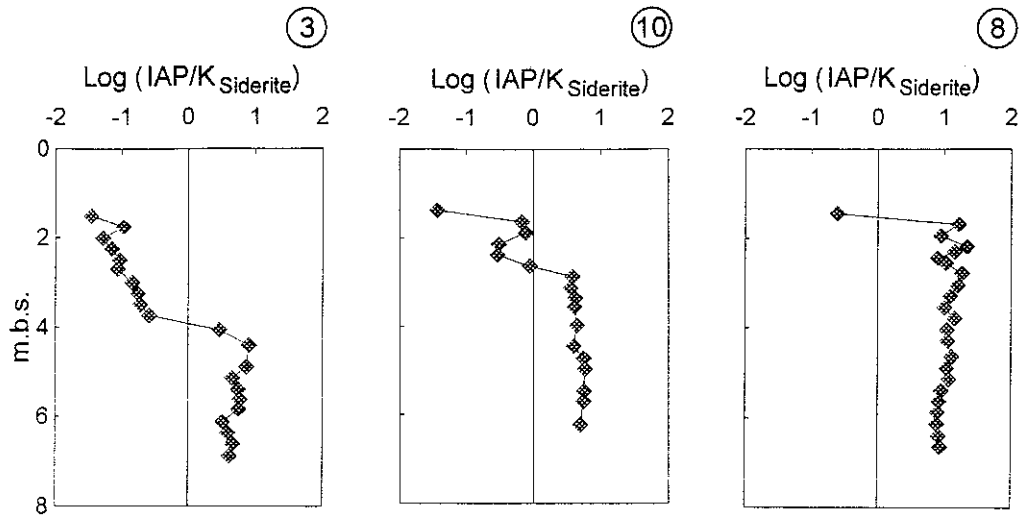

Figure 4.6. Saturation Index for siderite at three sampling locations in the Rømø aquifer (encircled numbers). Details of the calculations are found in the text.

The water in the Rømø aquifer was about 5-15 times supersaturated with siderite below 4 m.b.s. at location 3, below 3 m.b.s. at location 10 and at all but the shallowest depths at location 8 in July 1997. Probably siderite precipitated and the Fe^{2+} concentrations in these parts of the aquifer must be controlled mainly by that reaction.

At locations 3 and 8 the sulfate concentrations decreased almost linearly in 1996 and reached very low levels (1-20 µM) within just 1 m below the groundwater table. This decrease in sulfate concentration occurred concurrently with the increase in Fe^{2+} concentration, indicating sulfate reduction to be occurring concurrently with Fe(III)-oxide reduction in this part of the aquifer. A typical example of this is provided by a close up on the redox chemistry in the upper part of the aquifer at location 3, shown in figure 4.11. At location 10 the picture was somewhat different in December 1996. No sulfate was present in the top of the aquifer, however, as previously mentioned, some sulfate (< 0.05 mM) was found 3 m.b.s.

In general the sulfate concentrations increased significantly from October/December 1996 to July 1997. The maximum sulfate concentration increased mainly at location 3 (from 0.15 to 0.5 mM) and at location 10 (from 0.05 to 0.25 mM). At location 10 sulfate was found in July 1997 at the most shallow depths rather than at intermediate depths as in December 1996. At location 8, the major change was, that the highest sulfate concentration in July 1997 was found as a narrow peak at 2 m.b.s., rather than in the top of the profile as in December 1996. Obviously some water with quite high concentrations of sulfate infiltrated the aquifer during the winter 1996-1997.

When sulfate concentrations were measured in water centrifuged out of the sediment collected for sulfate reduction rate measurements, even higher concentrations of sulfate (and chloride) were found at location 10. This is somewhat puzzling, since the sediment was collected less than ½ m from where the water samples were taken and only 1-2 weeks later. The measured concentrations of sulfate and chloride in normal water samples and water centrifuged out of sediment are shown in figure 4.7.

It was investigated whether one or both sets of measurements were influenced by systematic errors, but none were detected, and a repeated measurement of the normal water samples gave similar results (no sediment was left to enable a repetition of that analysis). Moreover, the differences between the sulfate and chloride concentrations at the other locations, measured by the same methods, were minor. Therefore the sulfate and chloride concentrations must actually have been significantly higher in the core taken for sulfate reduction rate measurements at location 10 than in the water samples. This must then reflect a very sharp front of infiltrating water with a high ionic strength somewhere upstream from location 10 during the winter 1996/1997, which coincidentally was captured by these measurements. The source of the high ionic strength water could for instance be a high tree catching large amounts of dry deposition during the summer.

The molar relation between sulfate and chloride at location 10 and 8 are up to 0.06-0.13 in the upper part of the aquifer. These values are higher than the ratio found in seawater (0.045) but are similar to those previously measured in the Rømø aquifer (Jakobsen, 1995; Larsen, 1998). The reason for the high SO_4^{2-}/Cl^- relation is most likely an increased concentration of sulfate in rainwater, due to the oxidation of sulfur compounds from natural and/or anthropogenic sources in the atmosphere (Savoie & Prospero, 1989; Bates et al. 1992). At location 3 the molar ratio between SO_4^{2-} and Cl^- was up to 0.33 in the upper part of the aquifer in July 1997. Moreover, while the concentration of sulfate increased significantly here from October 1996 to July 1997, the concentration of chloride remained practically constant (figure 4.7). For this reason it is not likely, that the high sulfate concentration at location 3 in July 1997 was derived primarily from rainwater.

A possible explanation is, that a major lowering of the groundwater table, occurring in the summer of 1996, caused pyrite oxidation to occur in the newly formed unsaturated zone. 1996 was one of the driest years ever recorded in Denmark, with an exceptional low infiltration. As will be shown in section 4.4.1, sulfate reduction occurs very close to the groundwater table at the sampled locations. Pyrite, formed by sulfate reduction during the winter, could therefore be oxidized, when the groundwater table is lowered during the summer. This is possibly the process responsible for the large sulfate enrichment in the water at location 3 in July 1997, as well as for some of the general sulfate enrichment. Fe^{2+} concentrations did not increase in the upper parts of the aquifer at location 3 from October 1996 to July 1997. Therefore, if pyrite oxidation is responsible for the

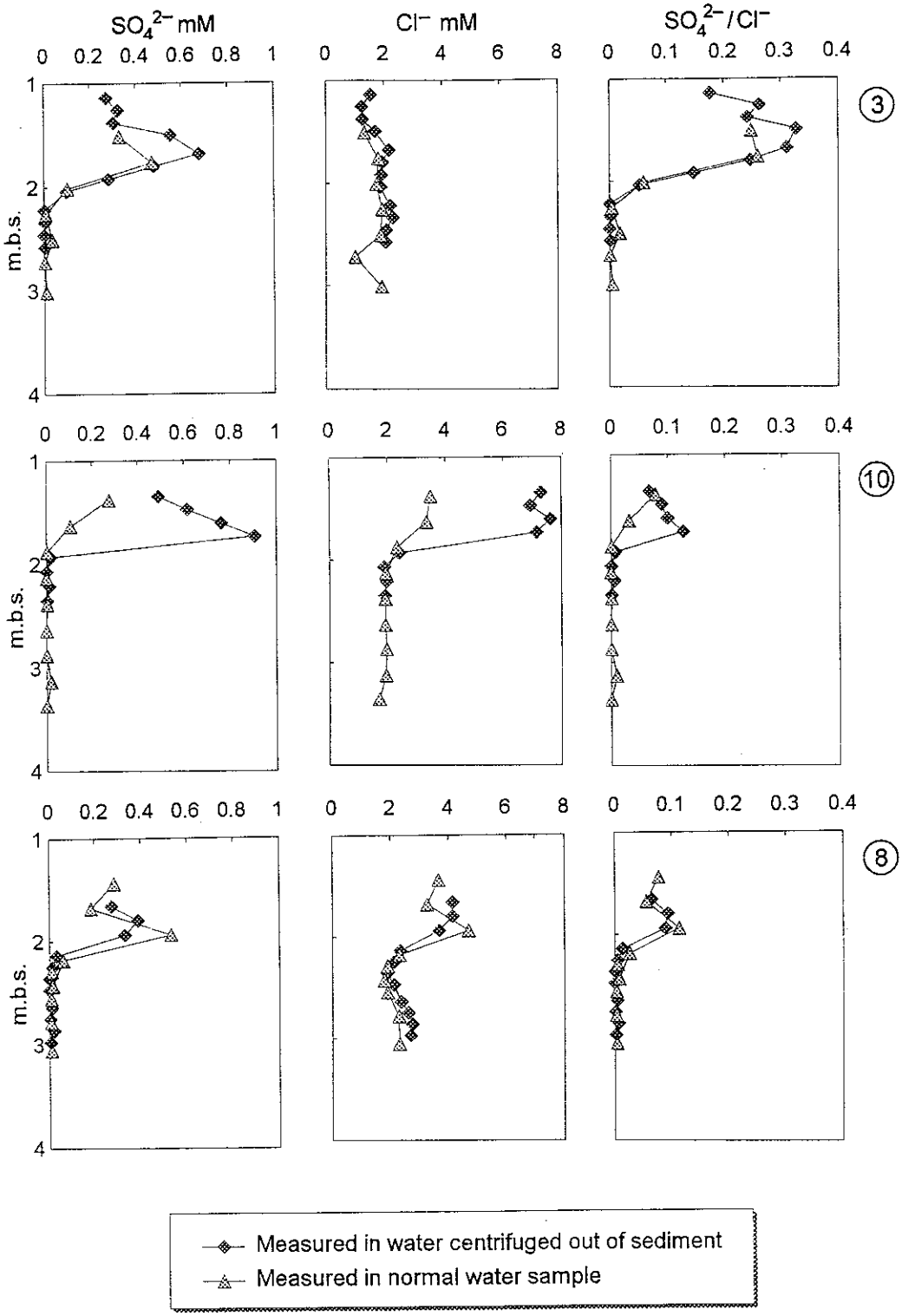

Figure 4.7. Concentrations of sulfate and chloride measured in normal water samples and in water centrifuged out of sediment at three sampling locations (encircled numbers) in the Rømø aquifer. Also shown is the molar relation between SO_4^{2-} and Cl^-. All samples were taken in July 1997.

increased concentration of sulfate in July 1997, then it must be complete pyrite oxidation, where Fe^{2+} is oxidized to Fe^{3+} and precipitated as Fe(III)-oxides.

The extreme weather pattern of 1996 might also explain why the water at location 10 was almost completely depleted for sulfate in December 1996. If sulfate reduction occurs fast, as indicated by the concentration profiles (figure 4.5 and 4.7) and by the radiotracer rates (figure 4.15), then a complete depletion of sulfate can occur after a long period with little or no infiltration.

At all three locations methane starts to build up within less than 1 m below the groundwater table. Some overlap between sulfate and methane containing water does occur, as shown by the close up of the upper part of the aquifer in figure 4.11. However appreciable concentrations of methane are not found until the water is depleted for sulfate. In the deep, sulfate free part of the aquifer, the concentration profiles for methane do not show a steady increase with depth, as would be expected, if methane production rates were temporally and horizontally homogeneous. Several local peaks are present, and at location 3 and particularly at location 8, the highest concentrations of methane are not found in the deepest part of the aquifer, but at intermediate depths.

The methane concentrations remained relatively constant at location 3 during the summer of 1996 (figure 4.5). In July 1997 however, the picture had changed a lot, with much higher methane concentrations from 2-5 m.b.s., and much lower concentrations from 6-7 m.b.s. Similar changes occurred at location 8 and 10 from December 1996 to July 1997. Even larger changes in the concentration of methane and sulfate were found, when some of the locations sampled by (Jakobsen, 1995) were resampled for this investigation. This is illustrated in figure 4.8 by examples from two sample locations.

At location 1 the boundary between sulfate and methane containing water was pushed downwards between June 1992 and August 1995, whereas the exact opposite happened at location 3 between April 1993 and July 1997. Apparently the redox zonation in the Rømø aquifer is very dynamic. When sulfate enters previously methane producing parts of the aquifer, sulfate reduction replace methane production. On the other hand, methane is readily formed in previously sulfate reducing parts of the aquifer, when sulfate is no longer present there. The depth concentration profile from August 1995 (location 1) deviates from all other profiles measured in the Rømø aquifer, since no methane was observed below the sulfate containing zone.

To check if upwards bubble transport of methane could affect the distribution of methane in the aquifer, the p_{CH4} was calculated for the July 1997 data using Henry's Law. The calculated p_{CH4} values are shown in figure 4.9 together with the calculated hydrostatic pressure. Bubble formation in water requires, that the sum of partial pressures for all dissolves gases (including water itself)

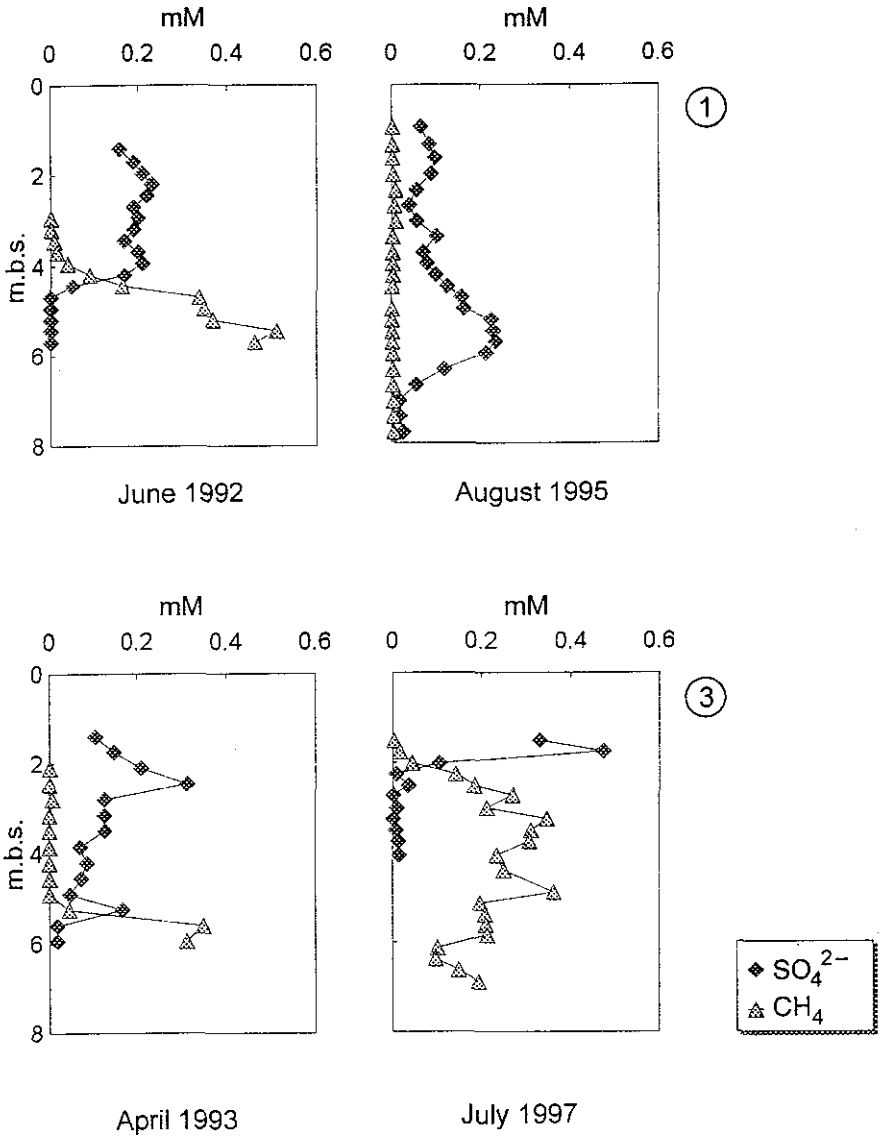

Figure 4.8. Changes in the concentrations of sulfate and methane over a period of 3-4 years at two sample locations (encircled numbers) in the Rømø aquifer. The data from 1992 and 1993 are from (Jakobsen, 1995), those from 1995 and 1997 are from this study.

exceeds the pressure in the water. In the Rømø aquifer, the dissolved gases are mainly H_2O, CH_4, CO_2 and N_2 + Ar. H_2O and CO_2 have partial pressures, that do not exceed 0.02-0.03 Atm. The partial pressure of N_2 and Ar cannot be exactly calculated, as concentrations of these gases were not measured. Since the input of nitrate is negligible, nearly all N_2 in the Rømø aquifer must have been dissolved in the water at the time of infiltration. This limits the possible range of partial pressures of N_2+Ar in the Rømø aquifer to 0.8-1.0 Atm, depending on the concentration of oxygen in the unsaturated zone.

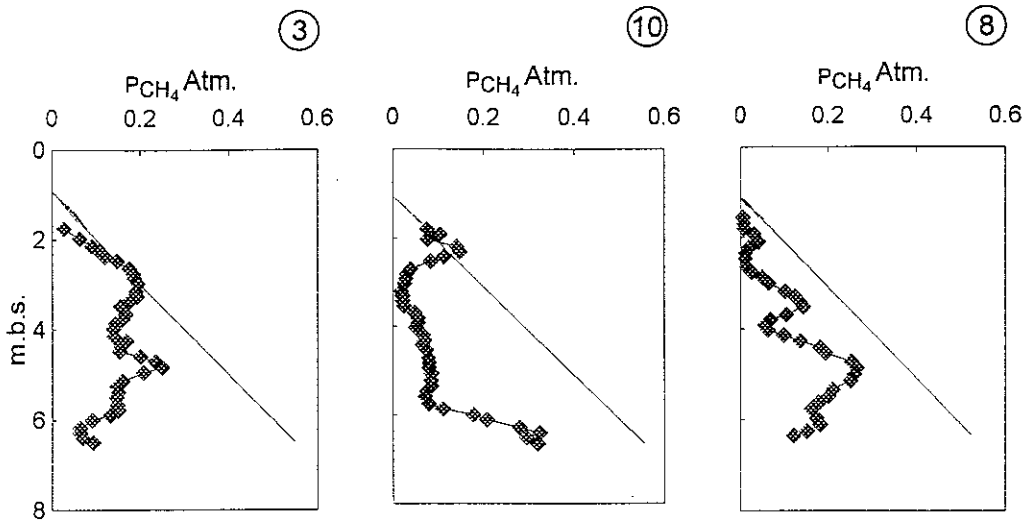

Figure 4.9 Calculated p_{CH4} in July 1997 at three sampling locations in the Rømø aquifer (encircled numbers). The solid lines represent the hydrostatic pressure.

A conservative assumption is, that the sum of partial pressures for all gases other than CH_4 does not exceed 1 Atm. The solid lines in figure 4.9, showing the hydrostatic pressure, are then the absolute minimum p_{CH4}, at which transport of CH_4 as bubbles could occur. Comparison with the measured data indicates that bubble formation cannot occur, since p_{CH4} is always below the hydrostatic pressure. There are though some small zones in the top of the aquifer at location 3 and 10, where p_{CH4} comes close to the hydrostatic pressure.

4.2.2 Fermentation products

As explained in section 1.1, it has been proposed by (Lovley & Goodwin, 1988) that hydrogen concentrations could be an indicator for TEAP. Using the same arguments, it is possible, that concentrations of other fermentation products could be used as another indicator for TEAP. For this reason, the concentrations of hydrogen as well as the short chain fatty acids formate and acetate were measured at the three sampling locations. The results are shown in figure 4.10.

At all three locations, the concentration of H_2 is in the range 0.2-0.4 nM in the uppermost part of the aquifer, which according to (Lovley & Goodwin, 1988) indicate Fe(III)-oxide reduction. At location 10 and 8, the H_2 concentrations increases gradually with depth and reaches a level of ~1 nM, which should be indicative for sulfate reduction. At location 3 the increase in H_2 concentration occurs rather abruptly around 2 m.b.s. Below 2 m.b.s. the H_2 concentration is generally in the 1-3 nM range indicating sulfate reduction, but a few measuring points show more than 3 nM H_2. However the concentration range for methanogenesis (7-10 nM) is never attained.

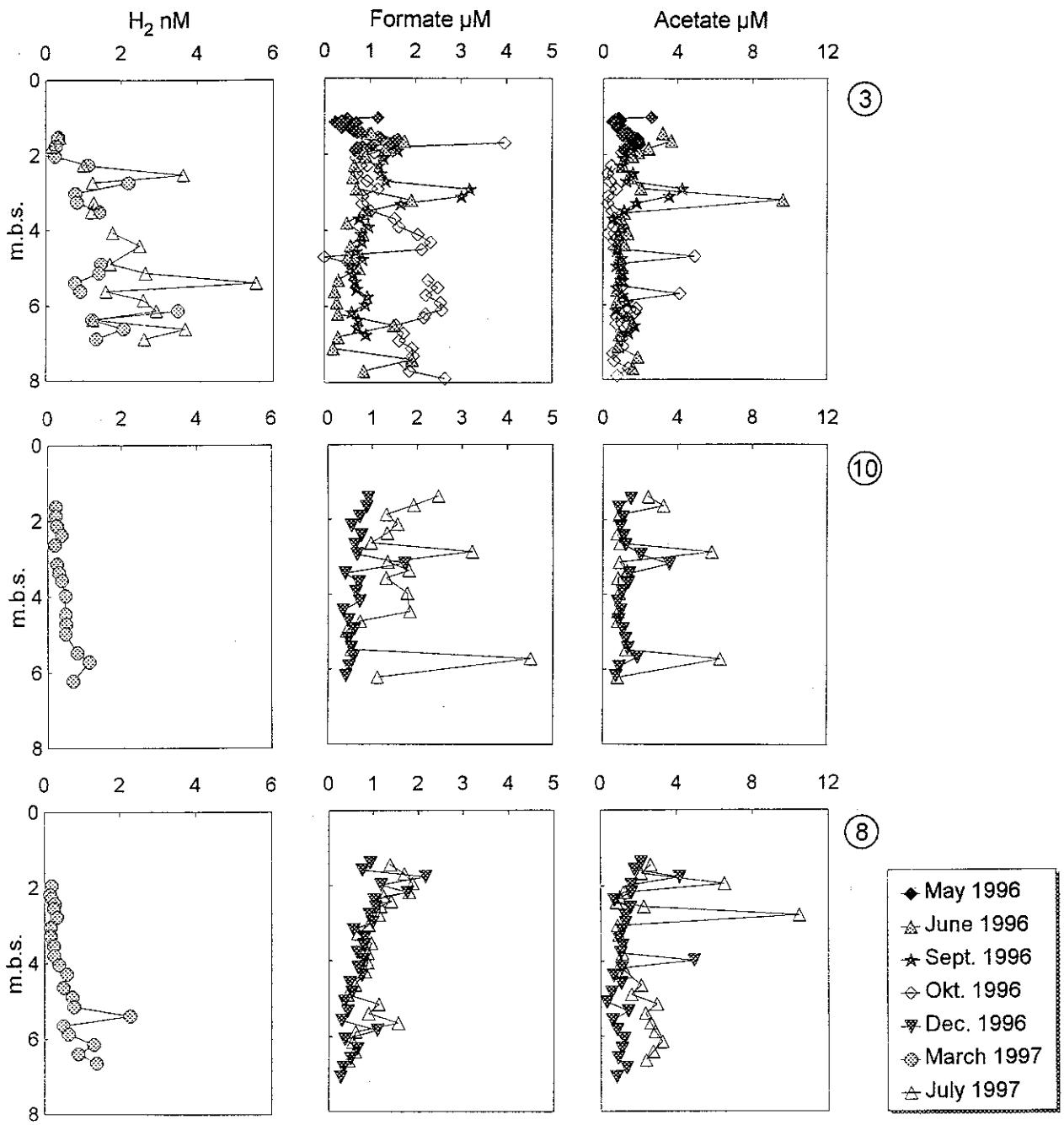

Figure 4.10. Concentrations of H_2, formate and acetate at three sampling locations (encircled numbers) in the Rømø aquifer.

Except from a few measuring points, the two sets of measurements from location 3 show similar H_2 concentrations, which indicate that temporal changes in the H_2 concentration are not important in the Rømø aquifer, as long as the dominating TEAP remains the same.

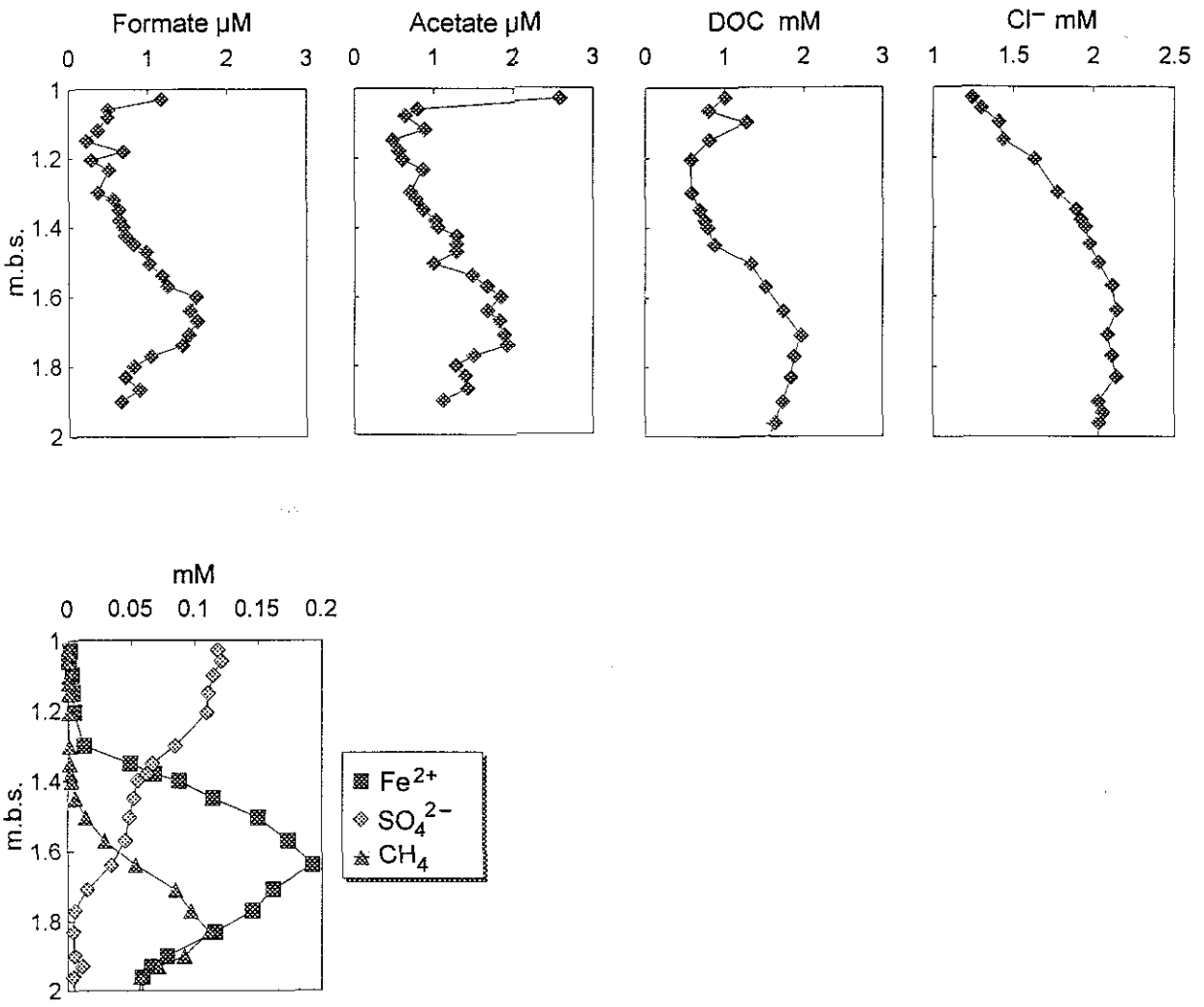

Figure 4.11. Closeup on the redox chemistry, DOC and acetate/formate concentrations in the upper part of the Rømø aquifer at location 3. The samples were taken in May 1996.

The concentrations of formate and acetate (figure 4.10) deviates considerably from the H_2 concentrations. Firstly they are about 3 orders of magnitude higher than the H_2 concentrations. A general level of 0.5-1.0 µM for formate and 0.7-1.5 µM for acetate is found at all three locations. Moreover, while the H_2 concentration generally increases with depth, this does not occur for formate nor acetate. Apparently there is no simple correlation between the H_2 concentration and acetate/formate concentration. Similar observations have been made by others. (Vroblesky et al., 1997) analysed numerous samples from an oil polluted aquifer and found no correlation between H_2 concentrations and organic acids concentrations.

The acetate and formate concentration profiles have several narrow peaks, where up to 10 µM acetate and up to 5 µM formate is found. These peaks are often not found again, when sampling

is repeated. Possibly they represent temporary nonequilibrium or very local conditions. But many of these peaks are indicated by one measuring point only, and it can therefore not be ruled out, that some of them could be outliers resulting from individual pollution of samples.

The general level of formate and acetate concentrations seems to be more constant over time, but some variations are nonetheless found.

Sometimes a change in concentration is observed for both organic acids, but just as often this is not the case. There is therefore no simple correlation between the concentration of formate and acetate either.

Some or all of the changes in the formate and acetate concentrations between the different sampling times might be due to small scale horizontal variations in the sediment. As explained in section 2, repeated sampling was done at a distance of 0.5-1.0 m from the original sampling locations. Given a horizontal flow velocity of 10 m/yr., advective transport of organic acids over such distances would take about 450-900 hours. Since the turnover time for acetate in the Rømø aquifer varies from 5-500 hours (figure 5.4), this means, that substantial differences in the acetate concentrations could theoretically exist between sampling points located just 0.5-1.0 m apart.

4.2.3 Ammonium

Ammonium is formed as a byproduct in the decomposition of organic matter. Concentrations of ammonium at the three sampling locations are shown in figure 4.12.

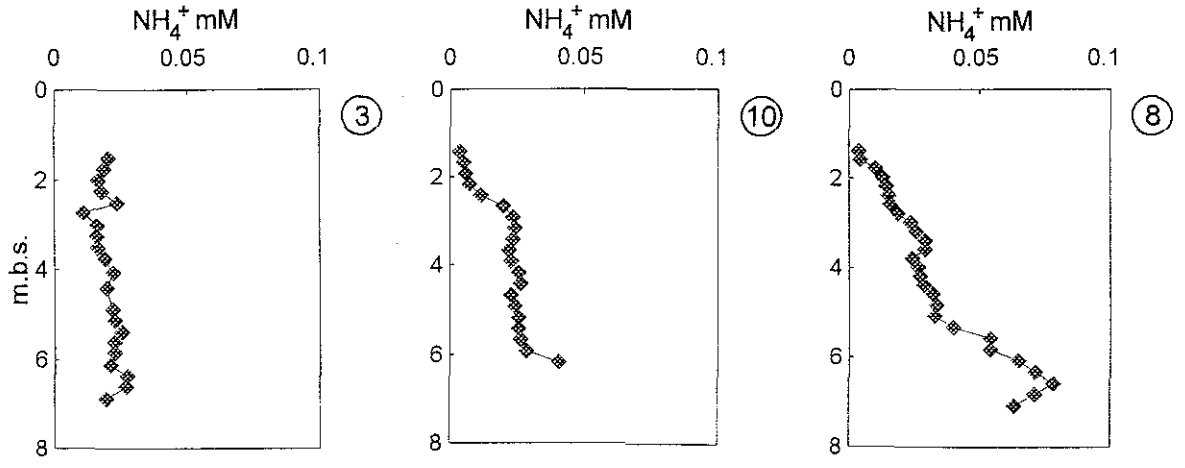

Figure 4.12. Concentrations of NH_4^+ at three sampling locations (encircled numbers) in the Rømø aquifer. The samples were taken in December 1996 (location 10 and 8) and in July 1997 (location 3).

The concentration of NH_4^+ increases with depth at location 10 and 8, as would be expected if decomposition of organic matter by redox processes occurred. This is not the case at location 3, where the NH_4^+ concentration is nearly constant around 0.02 mM. The highest NH_4^+ concentrations are found at the base of location 8, where a level of 0.08 mM is reached. If a constant content of N in the decomposed organic matter can be assumed, then it should be possible to correlate directly the NH_4^+ concentration to the amount of organic matter being decomposed. In an attempt to do this, the measured NH_4^+ concentrations were plotted against the concentration of CH_4, but this gave a very low correlation coefficient ($R^2 = 0.002$). Possible explanations for this could be, that there are large differences in the amounts of sulfate or Fe(III)-oxides that are being reduced along the different flow paths, that the N content in the organic matter being decomposed varies a lot or that the NH_4^+ concentrations were influenced by ion exchange. Or a combination of all these factors. Anyhow it seems difficult to obtain useful information from NH_4^+ concentrations in the Rømø aquifer.

4.3 Organic matter in sediment and water

To estimate the size of the different pools of organic matter, the content of DOC (Dissolved Organic Carbon) was measured at all three sampling locations. The content of SOC (Sedimentary bound Organic Carbon) was measured at location 3 only. The measured DOC values are shown in figure 4.13 The distribution of SOC at location 3, split up in two fractions, is shown in figure 4.14.

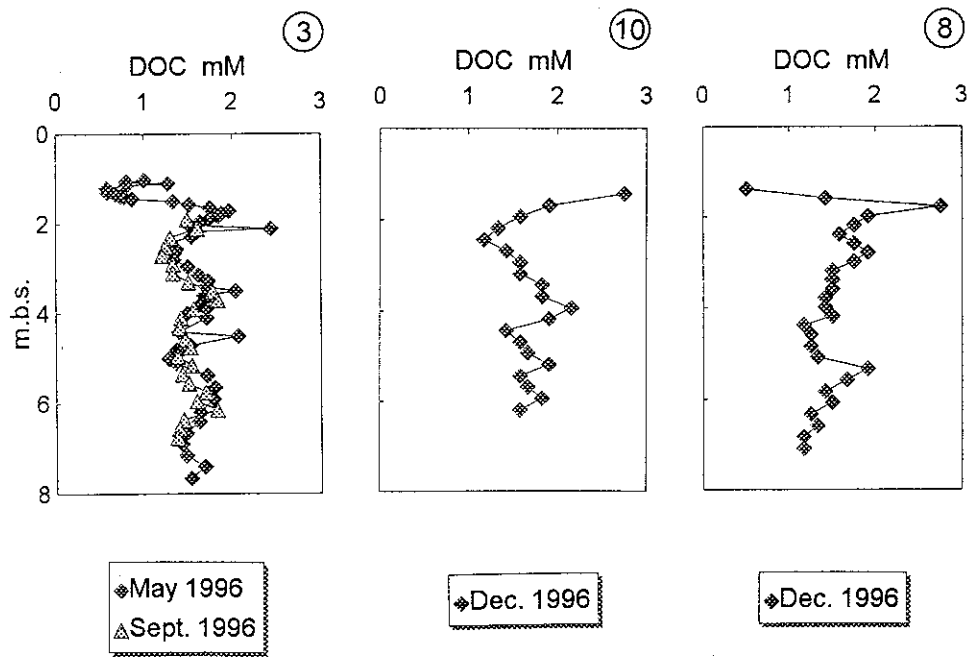

Figure 4.13. DOC in the Rømø aquifer. Encircled numbers refer to sampling locations.

The DOC profiles reveal some rather large fluctuations near the groundwater table. As shown in the closeup of the upper part of the aquifer in figure 4.11, these fluctuations seems to be at least partly correlated to the input of salt (chloride). Otherwise, there are no major variations in DOC over depth or between the three locations. Also, no major temporal changes were found, when location 3 was resampled after 4 month. A background level of 1-2 mM DOC exists at all three locations, which is relatively high for an aquifer. According to (Thurman, 1985), most aquifers have a DOC content of less than 2 mg/l (0.17 mM). The high DOC content in the Rømø aquifer is probably a result of the very shallow groundwater table (Starr, 1988). There is no decrease in DOC over depth, which indicate, that DOC is transported conservatively rather than being decomposed or adsorbed in the Rømø aquifer. Alternatively an eventual decomposition and/or absorption of DOC must be balanced by desorption of SOC.

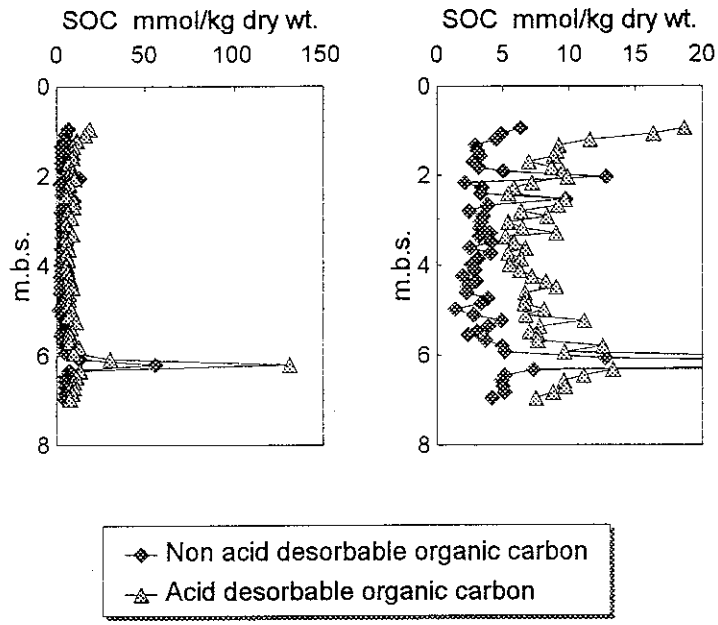

Figure 4.14. Acid desorbable and non acid desorbable SOC at location 3 in the Rømø aquifer shown with two different scales.

(Jakobsen, 1995) measured DOC values in the Rømø aquifer, that were quite similar to those found in this study. Only the range of values was larger (0.2-4.0 mM), perhaps because the sampled locations are situated over a larger area with more variation in the vegetation (figure 3.2). The lowest DOC values were all measured in water, that had infiltrated on the heath land. (Jakobsen, 1995) never measured a decreasing DOC with depth and actually DOC increased with depth in several of the profiles.

The content of organic carbon in the sediment at location 3 is generally quite constant around 10-15 mmol/kg dry weight. Since one litre of water is in contact with approximately 6 kg sediment, this means, that the SOC pool is about 40-60 times larger than the DOC pool. About one third of the SOC is non acid desorbable, the rest is acid desorbable. These values are similar to those measured by (Jakobsen, 1995) at location 1+2, but the total amount of SOC is slightly higher at

location 3. The only elevated SOC values at location 3 are found 1.0-1.3 m.b.s. and as a large but narrow peak 6.2 m.b.s. The sediment from 1.0-1.3 m.b.s. is submerged in the saturated zone for most of the time, but in dry years like 1996 the groundwater table is lowered to slightly below this depth. A large amount of root fragments was observed at this depth, which could contribute to the elevated SOC content.

The large but narrow peak in SOC 6.2 m.b.s. probably reflects a different sedimentary environment. In most of the sediment cores sampled at location 3, the sediment had a very different visual appearance around 6.2 m.b.s. being much darker and more fine grained than other sediment from the Rømø aquifer. When base was added to such sediment, the water turned nearly black, probably due to extraction of organic matter from the sediment. As shown in figure 4.4, there was also a very high content of SIC (both whole shells and shell fragments) in this sediment. The fine grain size and the correlation between the content of SOC and SIC in this sediment strongly indicates a marine origin.

The layer with a high SOC content around 6.2 m.b.s. was not observed in all cores from location 3 and may not have any large horizontal extension. Similarly looking thin (a few cm) layers of dark, fine grained, shell rich material, turning black by addition of base, were sometimes found also at other depths (always below 5 m.b.s.) at location 3 and in sediment from the deepest part at location 10.

To obtain qualitative information about the organic matter in the Rømø aquifer, HA`s (humic acids) and FA`s (fulvic acids) were isolated from water and sediment at four depths at location 3. The samples were taken in May-September 1996 and the isolations were carried out by the Plant Biology and Biogeochemistry Department, RISØ National Laboratory. The isotopic composition of the isolated FA`s and HA`s were determined by the AMS Laboratory, Institute of Physics and Astronomy, University of Aarhus. The results are shown in table 4.1

The dissolved FA`s and HA`s are modern at all depths and have $\delta^{13}C$ values in the -24 $^{o}/_{oo}$ to -28 $^{o}/_{oo}$, range, that is normally associated with terrestrial Calvin cycle plants (Spalding et al. 1978). Their origin must be the present soil zone, and old organic carbon, deposited with the sediment, contributes very little to dissolved FA`s and HA`s. This is different from other Danish aquifers, where similar characterizations of FA`s and HA`s have been carried out. In the Skagen and Fjand aquifers, the dissolved FA`s and HA`s originate from the sediment, and in the Tuse Næs aquifer, they originate partly from the sediment and partly from the soil zone (Grøn et al. 1996).

Depth: m.b.s.	Type of organic matter	^{14}C pmc	^{14}C Age (BP)	$\delta^{13}C(^{o}/_{oo})$ PDB	$\delta^{18}O(^{o}/_{oo})$ PDB
1.45	Sedimentary FA	106.9±0.6	-	-26.7	-20.7
1.45	Sedimentary HA	93.6±0.6	530±50	-26.8	-19.6
1.95	Sedimentary FA	105.0±0.6	-	-270	-20.6
1.95	Sedimentary HA	93.7±0.6	525±45	-25.9	-18.7
3.5	Sedimentary FA	104.4±0.6	-	-26.9	-20.6
3.5	Sedimentary HA	85.0±0.5	1305±50	-24.5	-18.8
6.5	Sedimentary FA	97.6±0.6	-	-25.5	-19.5
6.5	Sedimentary HA	73.9±0.5	2425±50	-23.1	-19.1
1.45	Dissolved FA	111.7±0.5	0	-270	
1.45	Dissolved HA	111.0±0.5	0	-26.7	
1.95	Dissolved FA	116.3±0.5	8	-27.5	
1.95	Dissolved HA	115.8±0.5	7	-27.5	
3.5	Dissolved FA	117.8±0.5	10	-280	
3.5	Dissolved HA	113.2±0.6	3	-27.8	
6.5	Dissolved FA	119.0±0.5	10	-27.8	
6.5	Dissolved HA	118.9±0.5	10	-27.9	

Table 4.1. Isotopic composition of FA`s and HA`s in sediment and water from location 3 in the Rømø aquifer.

If DOC moved conservatively through the aquifer, it should be possible to date the groundwater by the ^{14}C content of DOC, using the post bomb content in the atmosphere as a calibration curve. This was attempted, and as shown in table 4.1, the uppermost sample has the expected age of zero years. At larger depths however, most of the samples indicate groundwater ages, that are somewhat larger than expected from the vertical flow velocity of 1.25 m/yr. (section 3). A possible explanation is, that DOC, leaching from the soil zone, is retained to some extend in the aquifer through reversible adsorption to the sediment. The values shown in table 4.1 does however not indicate any simple retardation factor, since the ^{14}C ages of FA`s and HA`s from 1.95 m.b.s. are almost identical to those of FA`s and HA`s from 6.5 m.b.s. It is also puzzling, that there is a large difference between the age of FA`s and HA`s at 3.5 m.b.s. but not at the other depths. It can therefore not be ruled out, that other factors than a simple retardation of FA`s and HA`s could be responsible for the measured ^{14}C contents.

The ^{14}C ages determined for sedimentary HA`s are very similar to the expected age of the sediment: around 500 years in the top of the aquifer, increasing to 2400 years 6.5 m.b.s. This indicates, that the sedimentary HA`s are remnants of the organic matter, that was deposited with the sediment. The FA`s in the sediment have calculated $^{14}C$ ages, that are considerably younger than the HA`s. The ^{14}C content in all four samples are however significantly below the values, that existed in the atmosphere in 1996 (about 111 pmc), and it decreases with depth. This indicates, that the FA`s in the sediment are a mixture of modern organic carbon from the soil zone and older organic carbon. The sediment has been exposed to organic matter, leaching from the soil zone, for several houndred years. Therefore, the young age of the FA`s adsorbed to the sediment show, that the part of them, that is derived from the soil zone must be turned over by desorption or decomposition within a few years.

The $\delta^{13}C$ values found in FA`s from the sediment are quite constant over depht and all values are in the -24 $^{o}/_{oo}$ to -28 $^{o}/_{oo}$ range, indicating a terrestrial origin (Spalding et al. 1978). Only in the sample from 6.5 m.b.s. is a somewhat lower $\delta^{13}C$ value found, which might indicate some content of marine FA`s in this sample. In contrast the $\delta^{13}C$ values for sedimentary HA`s increase consistently with depth. In the sample from 6.5 m.b.s. the $\delta^{13}C$ value even fall in the -22 $^{o}/_{oo}$ to -24 $^{o}/_{oo}$ range, that is typical for marine organic matter (Nissenbaum & Kaplan, 1972). This further supports, that the sediment from the lower part of the aquifer was deposited in a marine environment. The $\delta^{13}C$ value found in sedimentary HA`s from 3.5 m.b.s. is also pretty close to the marine range. Perhaps this sediment was deposited at the upper edge of the wide marine foreland, that still exists on the Rømø Island, or slightly behind it in an area, that was occasionally flushed by the sea. In such a sedimentary environment, it is perfectly logical, that the organic matter deposited is partly of marine and partly of terrestrial origin.

4.4 Rates of redox processes

While the concepts, that were described in the previous section, might give useful information about the occurrence of redox processes, much more reliable and detailed information can be obtained by measuring the rates of the redox processes directly, using radiotracers (section 1.4). Sulfate reduction rates, methane production rates, acetate turnover rates and methane oxidation rates were therefore measured by the methods described in section 2.5.

4.4.1 Sulfate reduction rates

The measured sulfate reduction rates are shown in figure 4.15 together with the concentrations of SO_4^{2-} and Cl^-, that were measured in water from the cores used for the rate measurements.

Sulfate reduction takes place in the upper, sulfate containing part of the Rømø aquifer at all three locations. The highest sulfate reduction rates varies from 1.3 mM/yr. at location 8 to 2.8 mM/yr.

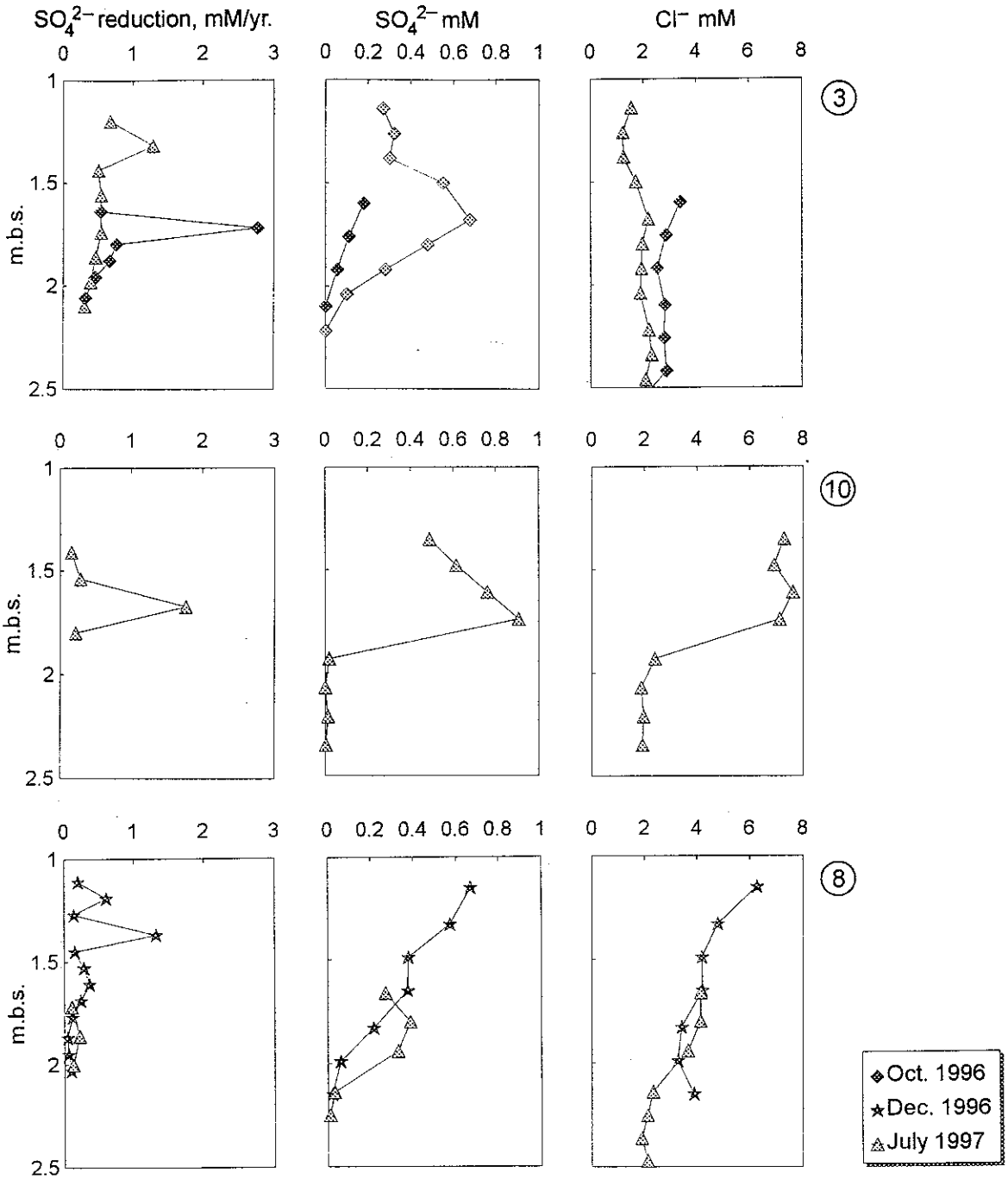

Figure 4.15. Sulfate reduction rates and corresponding concentrations of SO_4^{2-} and Cl^- at three sampling locations in the Rømø aquifer (encircled numbers).

at location 3 and there are very abrupt changes in the rates over depth. All major peaks in the rates are indicated by one measuring point only, despite the fact, that the spacing between samples is only 8-17 cm. No major temporal fluctuations in the sulfate reduction rates are revealed. Repeated sampling at location 3 and 8 gave almost identical results, except for a single measuring point at location 3, indicating a very high rate of 2.8 mM/yr. Since the sulfate concentrations increased

significantly from October/December 1996 to July 1997, this indicates, that the sulfate concentration does not have any large influence on the sulfate reduction rates in the Rømø aquifer. Other factors, like the reactivity of organic matter, must be rate limiting. This is in accordance with the findings from other low-sulfate freshwater sediments, where the half-saturating sulfate concentration has been determined to something like 0.05-0.10 mM (Roden & Tuttle, 1993). Since the sulfate concentration is well above this level in most parts of the sulfate reducing zone, it is reasonable, that the sulfate reduction rates are largely independent on the sulfate concentration.

A comparison between the measured sulfate reduction rates and the rates, that can be calculated from the concentration depth profiles of sulfate, reveals no major discrepancy. A similar finding was made by (Jakobsen, 1995). Accordingly, the rate measurements seems to be reliable. However, while the general level of rates agrees well with the concentration profiles, much more details are disclosed by the directly measured rates. The rates measured in July 1997 are generally much lower than those, that could have been calculated from the concentration profiles by - erroneously - assuming a constant input of sulfate over time (figure 4.5 and 4.7). The rates are not constant over horizontal distances of more than a few metres either. At location 8 the rates measured 1.5-2.0 m.b.s. in both December 1996 and July 1997 are much too low to explain the decrease in sulfate concentration with depth. In agreement with this, sulfate was found at larger depth downstream from location 8 in December 1996 (data not shown). In conclusion, much more detailed and reliable information about sulfate reduction rates are obtained by measuring the rates directly, using a radiotracer.

4.4.2. Methane production rates

The measured methane production rates are shown in figure 4.16 together with the measured methane concentrations. Also shown in figure 4.16 is the calculated % of the total measured methane production, that occurred with acetate as the precursor. This was calculated by interpolation of the CO_2 reduction rates to the depths, where acetate fermentation rates had been measured.

At all three locations, methane production does not take place at appreciable rates, before sulfate is depleted from the water. This indicates, that sulfate reducing bacteria can outcompete methanogens for H_2 and acetate in the Rømø aquifer. At location 3 appreciable methane production begins right below the depth (2 m.b.s.), where sulfate is depleted from the water. At location 10 and 8 however, appreciable methane production does not begin until 1-3 m below the depth, where sulfate is depleted from the water. This indicates, that methanogens are being outcompeted for H_2 and acetate by a redox process other than sulfate reduction in the intervals 2-3 m.b.s. at location 10 and 2-4 m.b.s. at location 8. However, even when other electron

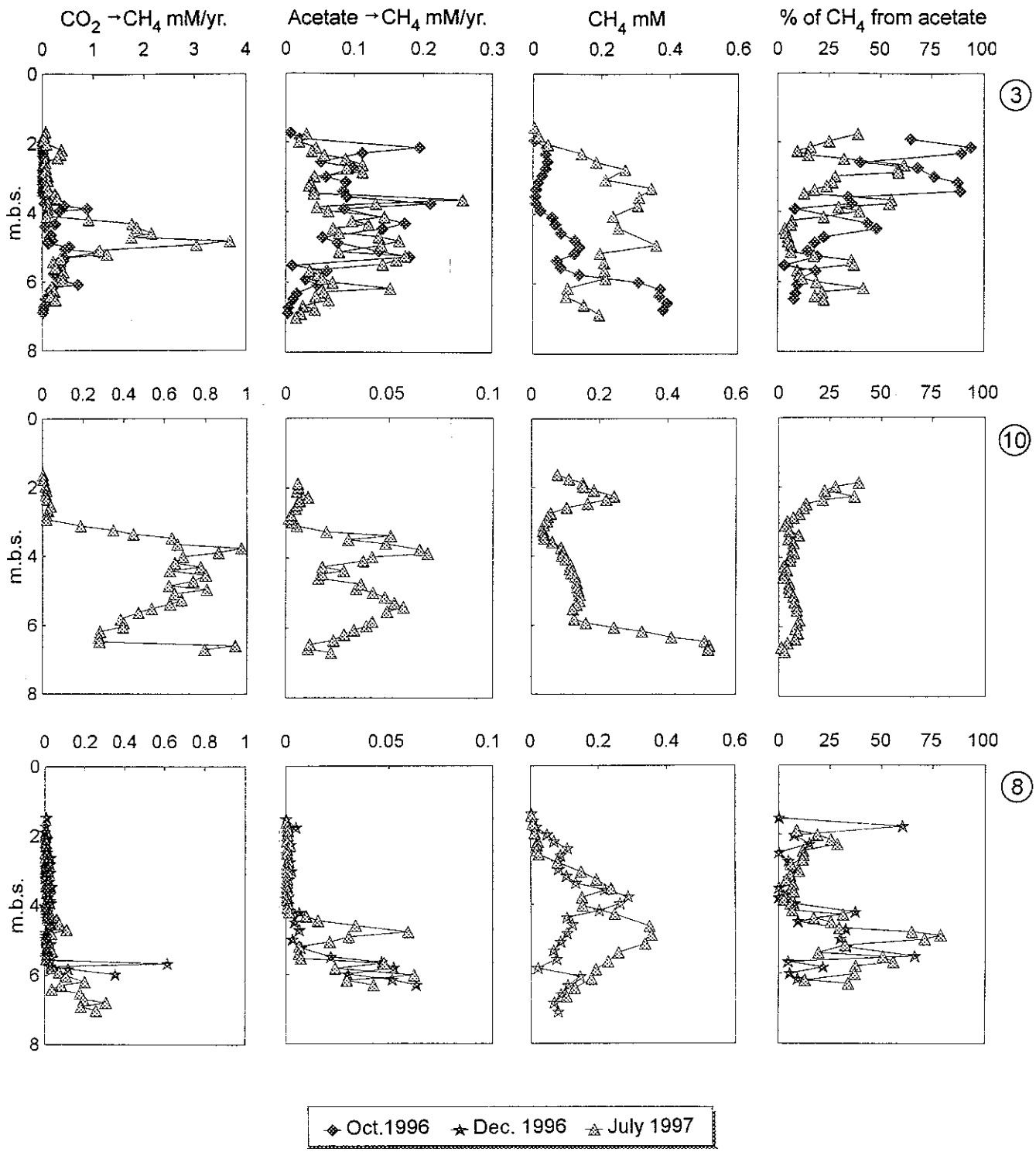

Figure 4.16. Methane production rates from H_2/CO_2 and from acetate, methane concentrations and % of methane production with acetate as the precursor at three sampling locations (encircled numbers) in the Rømø aquifer. Details on the calculations are found in the text. Note the different scales used for location 3. The rates from October and December 1996 were measured as part of a M.Sc.. thesis by (Nielsen & Holmefjord, 1997), but recalculated and redrawn by this author.

accepting processes dominate, low rates of methane production could always be measured. Methane production is therefore never completely excluded by other redox processes.

The total rate of methane production varies highly over depth and between the sampled locations. The maximum rates vary from 0.6 mM/yr at location 8 to 4 mM/yr at location 3. Overall, there is a large degree of covariation between the CO_2 reduction rates and the acetate fermentation rates. In particular, a high CO_2 reduction rate is never measured concurrently with a very low acetate fermentation rate or vice versa. Any competitive suppression of methane production must therefore occur concurrently for the CO_2 reduction and the acetate fermentation pathways.

In general, CO_2 reduction is the most important pathway of methane production in the Rømø aquifer. This is in accordance with the estimates in previous studies, that were based on the isotopic composition of methane (Coleman et al. 1988; Grossman et al. 1989; Aravena et al. 1995; Zhang et al. 1998). The relative importance of CO_2 reduction and acetate fermentation is far from constant, and in some parts of the aquifer, acetate fermentation is even the dominating process. Many of the small scale variations in the % of methane derived from acetate appears to be quite chaotic, and possibly some of these variations are simply the result of the slightly different sampling locations (cores were taken ½ m apart), minor errors in the determination of depths or perhaps analytical noise. A significant general trend is however, that when methane production rates are high (4-5 m.b.s. at location 3 and 3-5 m.b.s. at location 10), then only a minor part of the methane (5-10 %) is produced by acetate fermentation. At lower but still appreciable methane production rates, acetate is often a much more important substrate for methane, accounting for 20-90 % of the methane production.

The repeated sampling at location 3 and 8 showed almost identical acetate fermentation rates at most depths. There are some local peaks in the acetate fermentation rates though, that are present in one of the measurements but not in the other. Most noticeable is the peak around 5 m.b.s. at location 8 in July 1997. These local peaks could reflect temporal but also horizontal differences in the rates, since the cores were taken about ½ m apart. Still, when the overall picture is considered, the acetate fermentation rates were essentially the same at the different times of sampling.

The same cannot be said about the CO_2 reduction rates. There are some parts of the aquifer, where the CO_2 reduction rates were essentially the same at the two times of sampling, but there are large differences in other parts of the aquifer. Most profound is the very large peak in the CO_2 reduction rate at location 3, 4-5 m.b.s., that was found in July 1997 but not in October 1996. Also at shallow depths (2-4 m.b.s.) was the CO_2 reduction rate at location 3 much higher in July 1997 than in October 1996.

The methane production rates measured with the radiotracers deviate a lot from those, that can be calculated from the concentration depth profiles of methane. In the upper part of the aquifer, the measured rates are generally much lower than the calculated, and in the deeper part of the aquifer, they are often much higher (location 3 and 10). The concentration depth profiles even indicate negative rates (decreasing concentration with depth) in parts of the aquifer, where methane production takes place at high rates. This could theoretically be an effect of methane oxidation, but as will be shown in the following section, this is not the case. Accordingly, the methane production rates must be highly variable, temporally and/or horizontally, in the Rømø aquifer, and a direct measurement of the rates is necessary to get a reliable picture of where in the aquifer, methane production occurs.

4.4.3 Acetate turnover rates

As explained in section 1.4, the ^{14}C-methyl group in 214C-acetate should end up as $^{14}CH_4$, if 2-14c acetate is fermented by methanogenic bacteria. If, on the other hand, 2-^{14}C-acetate is oxidized by other bacteria than methanogens, the ^{14}C-methyl group should end up as $^{14}CO_2$. The measured rates of acetate oxidation and fermentation are shown in figure 4.17. Also shown in figure 4.17 is the respiration index of acetate, RI, defined by e.g. (Sansone & Martens, 1982) as:

(8)

$$RI = \frac{[^{14}CO_2]}{[^{14}CO_2] + [^{14}CH_4]}$$

where $[^{14}CO_2]$ and $[^{14}CH_4]$ are the activities of $^{14}CO_2$ and $^{14}CH_4$ formed from 2-^{14}c-acetate during the incubation.

Figure 4.18 shows a cumulative plot of the rates of acetate oxidation and fermentation, where a smaller y-axes scale gives a better overview of the rates in the deeper part of the aquifer.

The rates of acetate oxidation are very high at the most shallow depths in the aquifer, where sulfate reduction (figure 4.15) and Fe(III)-oxide reduction (indicated by the Fe^{2+} concentration profiles, figure 4.5) takes place. The acetate oxidation rates measured in the sulfate containing zones at location 3 and 8 are similar to or even higher than the sulfate reduction rates measured at the same depths. Some acetate is probably also being oxidized by Fe(III) reducers at these depths, but nonetheless this indicates, that acetate is a very important precursor for sulfate reduction in this part of the aquifer, since one mole of acetate is necessary for the reduction of one mole of sulfate. At location 10 no measurements of acetate oxidation rates were made in the sulfate containing zone, so the same comparison cannot be made there.

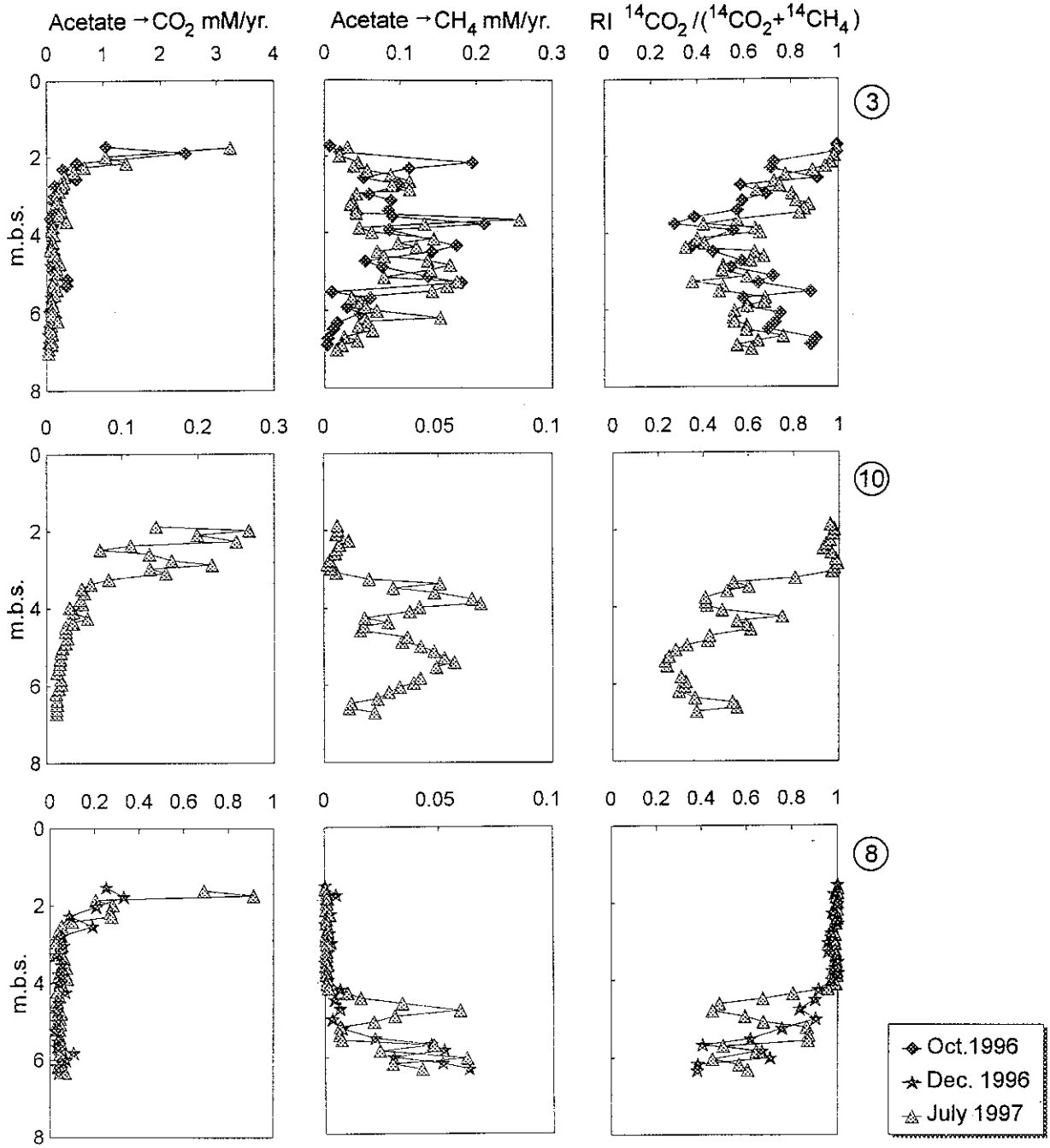

Figure 4.17. Rates of acetate oxidation and fermentation and RI (see text) at three sampling location (encircled numbers) in the Rømø aquifer. Note the different scales used. The rates from October/December 1996 were measured as part of a M.Sc. thesis by (Nielsen & Holmefjord, 1997) but were recalculated and redrawn by this author.

Below the sulfate containing zone (2 m.b.s.), the rate of acetate oxidation is much lower and generally decrease with depth, except at location 8, where the rate is rather constant around 0.05 mM/yr. A few local peaks are found though, most profoundly at location 10, 3 m.b.s., where a high Fe^{2+} concentration (figure 4.5) was also measured in December 1996 and to a smaller extent

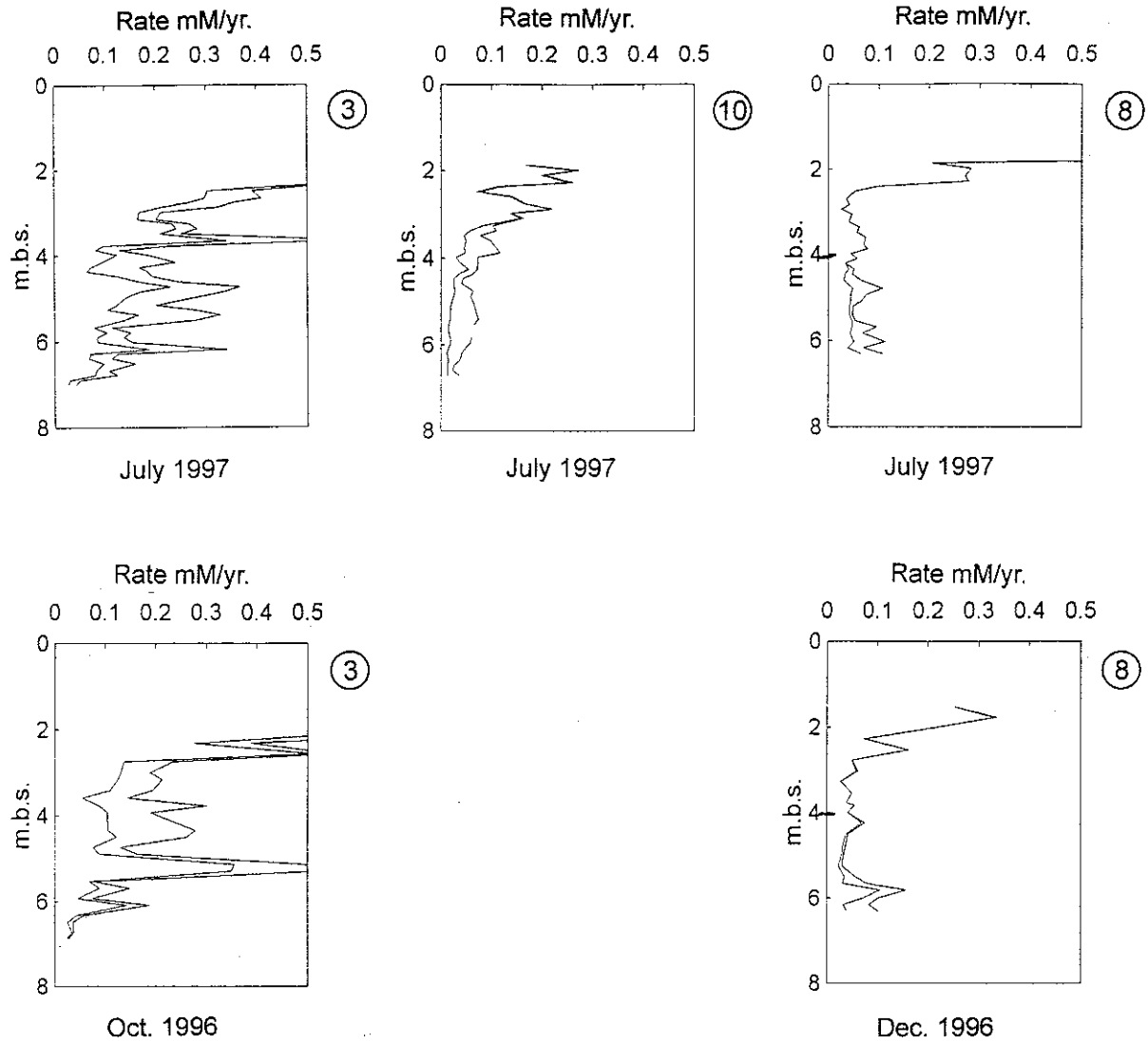

Figure 4.18. Cumulative plots of acetate oxidation (inner) and fermentation (outer) rates at three sampling locations (encircled numbers) in the Rømø aquifer. The rates from October/December 1996 were measured as part of a M.Sc. thesis by (Nielsen & Holmefjord, 1997) but were recalculated and redrawn by this author.

in July 1997, when the rate measurement was made. In the deeper parts of the aquifer (4-7 m.b.s.), the highest acetate oxidation rates are again found at location 3 (0.05-0.30 mM/yr.) and the lowest at location 10 (0.02-0.06 mM/yr.). In general, the acetate oxidation rate did not change much from October/December 1996 to July 1997. The only exception from this is the most shallow depths at location 8, where values up to 0.9 mM/yr. were found in July 1997 rather than the 0.35 mM/yr. found in December 1996.

As would be expected from the low methane production rates, only a minor fraction of the 2-^{14}c-acetate ended up as $^{14}CH_4$ in samples from the sulfate containing zone. The suppression of

methane production from acetate is strongest in the uppermost sample at location 8, where only 0.04 % of the acetate is turned over by methanogens. But even at depths down to 4 m.b.s., not more than ~1 % of the acetate is consumed directly by methanogens. At the other locations, the suppression of methane production from acetate in the upper part of the aquifer is not quite as strong, but still evident. At larger depths, methane production from acetate takes place at significant rates, and accordingly the RI decrease to 0.25-0.85 (figure 4.17). Still, 25 to 85 % of the acetate is oxidized rather than fermented in these parts of the aquifer.

4.4.4 Methane oxidation rates

As discussed in section 2.5, the measured methane oxidation rates seems to be hampered by systematic errors and are therefore only maximum estimates. The rates are shown in figure 4.19, together with the measured concentrations of methane and sulfate, the turnover of the injected $^{14}CH_4$ tracer during the incubations and the relation between methane oxidation and methane production rates. The latter was calculated by interpolation of the acetate fermentation and CO_2 reduction rates to the depths, where the methane oxidation rates were measured.

The methane oxidation rates are considerably lower than the rates of sulfate reduction and methane production. The highest rates vary from 0.017 mM/yr at location 3 to 0.07 mM/yr. at location 8, and these are even maximum estimates. So in general, methane oxidation is not a quantitatively important process in the Rømø aquifer. Unlike the findings from marine sediments (e.g. Iversen & Blackburn, 1981; Devil, 1983; Iverson & Jørgensen, 1985, Hoehler et al., 1994), there is no indication for sulfate being an important electron acceptor for the small amount of methane, that is being oxidized. The methane oxidation rates are very low in the sulfate containing part of the aquifer and increase considerably below the depth of sulfate depletion.

The turnover of injected tracer is relatively constant over depth at location 3 and 10 and the methane oxidation rates are therefore mainly controlled by the methane concentration here. This might indicate, that the apparent methane oxidation measured here is mainly the result of analytical artefacts: a tiny bit of $^{14}CO_2$ is always found in the analysis (figure 2.5). At location 8, on the other hand, the turnover of tracer is generally larger and there seems to be some systematic variations over depth, that might indicate, that we are closer to measuring real rates. There is however still the problem, that the finding of $^{14}CO_2$ is largely independent of the finding of $^{14}CH_4$, even at location 8 (figure 2.5). This discrepancy remains unexplained. Despite the systematic error introduced by this artefact, it is evident, that the rate of methane oxidation is largely independent of the rate of methane production. Where the methane production rates are high (4-5 m.b.s at location 3 and 3-5 m.b.s. at location 10) < 1 % of the produced methane is being reoxidized, whereas a much larger fraction of the produced methane is being reoxidized in the parts of the aquifer, where methane has been transported into areas with low methane production rates (2-3

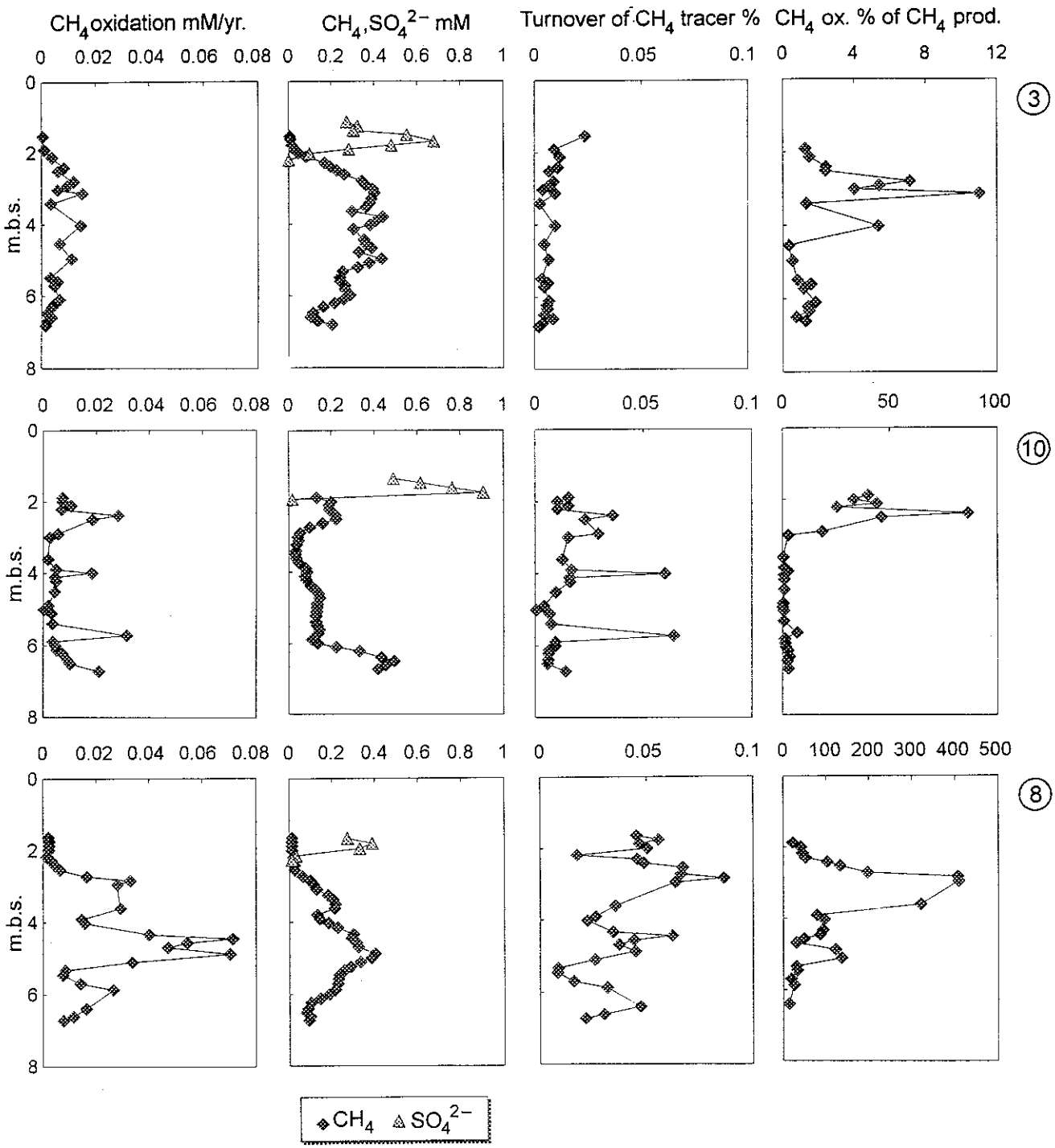

Figure 4.19. Maximum methane oxidation rates at three sampling locations (encircled numbers) in the Rømø aquifer, concentrations of methane and sulfate, turnover of injected $^{14}CH_4$ tracer and relation between methane oxidation and methane production. Details on the calculations are found in the text.

m.b.s. at location 10 and 2-4 m.b.s. at location 8). At location 8 net methane oxidation even seems to be taking place, but due to the previously mentioned problems, the evidence for this is highly uncertain.

4.5 Isotopic composition of methane and TIC

As discussed in section 1.2, the isotopic composition of methane might be used to deduce the dominant pathway of methane production and to determine whether a significant fraction of the methane has been reoxidized. The isotopic composition of methane is shown in figure 4.20. The samples were prepared by the Geological Survey of Denmark and Greenland and analysed by the Geological Institute, University of Copenhagen.

$\delta^{13}C_{CH4}$ varies from -50 $^{o}/_{oo}$ to -80 $^{o}/_{oo}$, the highest values being found in the upper part of the aquifer at location 8 and 11. These values are within the range normally found for bacterially produced methane, that is not significantly influenced by methane oxidation or by reservoir effects (Whiticar et al. 1986; Whiticar & Faber, 1986). δD_{CH4} is quite constant; values from -320 $^{o}/_{oo}$ to -300 $^{o}/_{oo}$ are found in the four samples analysed. These values are lower than those previously measured in deep aquifers (Coleman et al. 1988; Grossman et al. 1989; Aravena et al. 1995; Zhang et al. 1998). This indicate, that acetate fermentation is more important in the shallow Rømø aquifer than in deep aquifers. In the diagram proposed by (Whiticar et al. 1986, figure 1.3 in this text), the Rømø methane would even be classified as an acetate fermentation type.

Using the diagram proposed and used by (Aravena et al. 1995) for Canadian aquifers (figure 1.4), and assuming that δD_{H2O} is -70 $^{o}/_{oo}$, which is typical for Danish aquifers (Buchardt, 1998), we would conclude, that 40-50 % of the methane in the Rømø aquifer is produced by acetate fermentation and the remainder by CO_2 reduction. This is a slightly higher contribution of acetate fermentation than shown by the rate measurements. It should be remembered though, that the D/H fractionation factor for the methanogenic bacteria, on which the diagram in figure 1.4 is based, might be different from the D/H fractionation in the Rømø aquifer. For this reason, classification diagrams as those shown in figure 1.3 and 1.4 can only be approximations to a complex reality.

$\delta^{13}C_{TIC}$ and $^{14}C_{TIC}$ values were determined at location 3. The samples were analysed at the AMS Laboratory, Institute of Physics and Astronomy, University of Aarhus. The results are shown in table 4.2.

The increase in $\delta^{13}C_{TIC}$ over depth at location 3 is probably caused by calcite dissolution, that occurs below 4 m.b.s (figure 4.3). The values found above 4 m.b.s. indicates an organic origin of the TIC and the ^{14}C content is very close to the current content in the atmosphere (111 pmc). In contrast, the $\delta^{13}C_{TIC}$ values measured below 4 m.b.s. are between the values, that would be expected for organic carbon (-25 $^{o}/_{oo}$) and for dead carbonate (0 $^{o}/_{oo}$). Just as would be expected, if calcite dissolution occurred, releasing one mole of TIC form carbonate for each mole of organically derived CO2. At two depths (4.9 m.b.s. and 6.5 m.b.s.) the $\delta^{13}C_{TIC}$ values indicate a larger contribution from dead carbonate than from organic matter. This is not likely to be

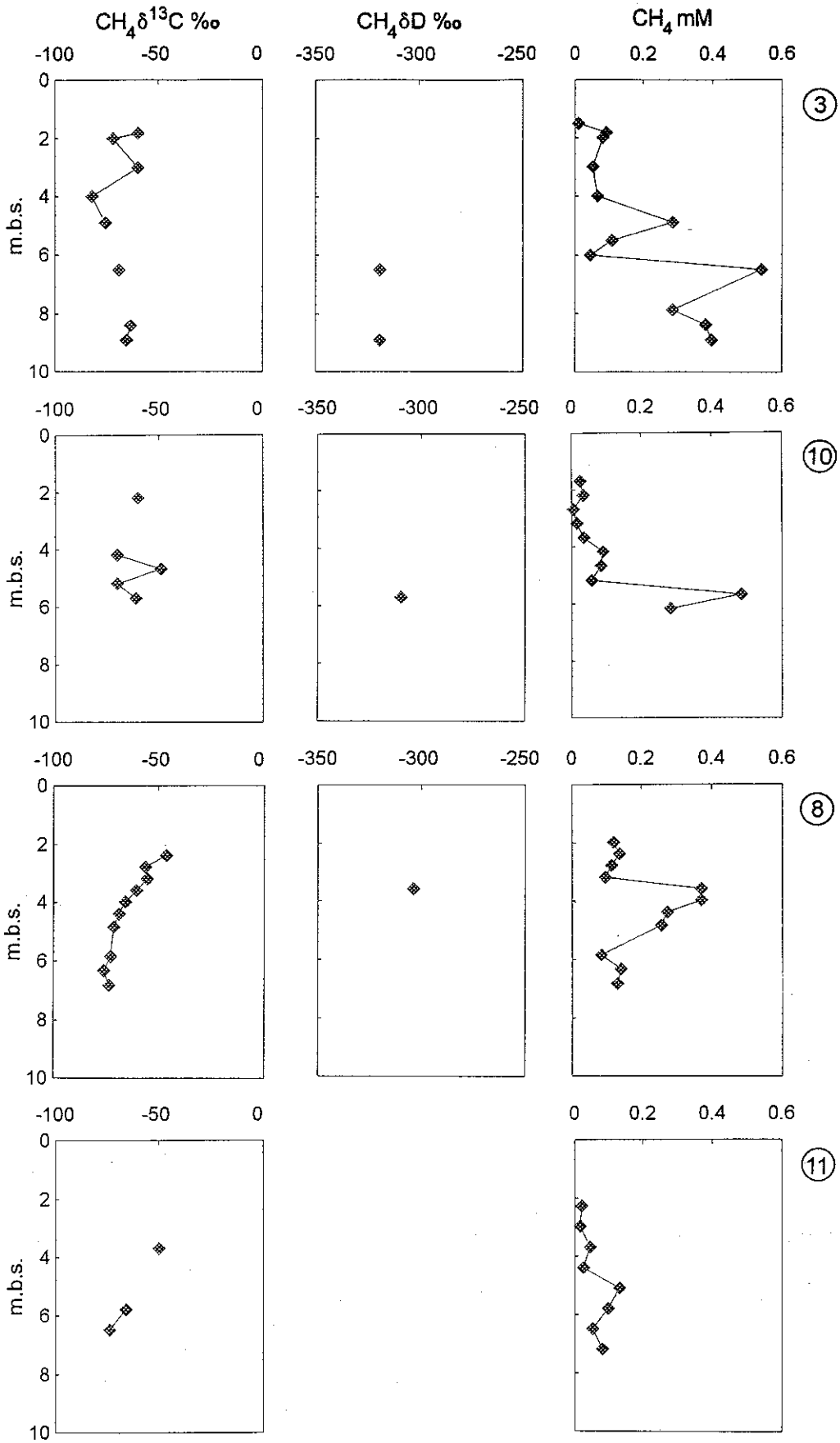

Figure 4.20. Isotopic composition and corresponding concentrations of methane at four sampling locations (encircled numbers) in the Rømø aquifer. The samples were taken in May 1996 (location 3) and December 1996 (location 10, 8 and 11).

correct, and it might reflect a pollution problem in these two samples. The ^{14}C content in all samples below 3 m.b.s. is also lower than would be expected from a simple mixture between modern carbonate from the soil zone and shell fragments. This could be a result of decomposition of old SOC, but it might also be a result of a polluted shell sample, since the ^{14}C content in HA`s from 6.5 m.b.s. was significantly lower (table 4.2).

Depth: m.b.s.	Parameter	$\delta^{13}C$ $^{o}/_{oo}$	^{14}C content: pmc
1.5	TIC(aq)	-21.3	113.3 ± 0.5
3.0	TIC(aq)	-17.6	114.6 ± 0.5
4.9	TIC(aq)	-9.5	88.4 ± 0.5
5.5	TIC(aq)	-13.0	89.9 ± 0.5
6.5	TIC(aq)	-8.8	90.3 ± 0.5
6.5	Shell fragments	-	81.1 ± 0.5
6.5	Sedimentary HA`s	-23.1	73.9 ± 0.5

Table 4.2. Isotopic composition of TIC in the Rømø aquifer. The samples were taken at location 3 in May 1996.

The increase in $\delta^{13}C_{TIC}$ caused by calcite dissolution is around 10 $^{o}/_{oo}$. Methane produced in calcite free parts of the aquifer should therefore be about 10 $^{o}/_{oo}$ more depleted in ^{13}C than methane produced in the calcite buffered parts of the aquifer, if the production pathways are the same. This does not seem to be the case, since the $\delta^{13}C_{CH4}$ values are independent of depth (location 3 and 10) or increase with depths (location 8 and 11), which is just opposite what one would expect from the $\delta^{13}C_{TIC}$ values.

It might be speculated upon, that the relatively high $\delta^{13}C_{CH4}$ values found at shallow depths at location 8 and 11 are the result of methane oxidation. However (Whiticar & Faber, 1986) found, that because of a low $^{13}C/^{12}C$ fractionation factor in methane oxidation, as much as 90-95 % of the methane had to be oxidized in marine sediments before a significant effect was seen on the $\delta^{13}C$ value. Given the high methane concentrations found at shallow depths at location 8, this already makes it unlikely, that methane oxidation could be responsible for the relatively high $\delta^{13}C_{CH4}$ values here.

5. Discussion

5.1 Rates of methane production, sulfate reduction and organic matter decomposition

The rates of sulfate reduction and methane production measured by radiotracers in the Rømø aquifer in this study and by (Jakobsen & Postma, 1994; Jakobsen, 1995) might be compared to those measured in marine and lake sediments with the same methods. In table 5.1 is shown some examples of maximum rates of sulfate reduction and methane production in sediments, measured by radiotracers. The rates were originally expressed in many different ways, but have been recalculated to mmol C/(litre sediment*year) to enable a comparison.

Site	TEAP	Maximum rate, mmol C/(l*yr)	Reference
Rømø aquifer	Sulfate reduction	3.2	(Jakobsen, 1995)
Rømø aquifer	Methane production	2.6	This study
FOAM (marine)	Sulfate reduction	80	(Berner, 1980)
C.L.B (marine)	Sulfate reduction	1100	(Crill & Martens, 1986)
C.L.B. (marine)	Methane production	180	(Crill & Martens, 1986)
Lake Washington	Sulfate reduction	1.1	(Kuivila et al. 1988)
Lake Washington	Methane production	2.6	(Kuivila et al. 1988)
Lake Woods	Sulfate reduction	0.8	(Rudd et al. 1986)
Lake Mendota	Methane production	700	(Phelps & Zeikus, 1985)

Table 5.1. Comparison of maximum rates of sulfate reduction and methane production in sediments. All rates were measured by radiotracers.

A wide span of sulfate reduction and methane production rates exists in sediments and the rates do not appear to be controlled by the salinity of the sediment. The rates in the Rømø aquifer are in the lower end of the range, but are similar to or higher than the rates in some lake sediments (Lake Washington and Lake Woods). On the other hand they are more than 2 orders of magnitude lower than the rates in Lake Mendota and Cape Lookout Bight (C.L.B.).

In contrast, the rates in the Rømø aquifer are orders of magnitudes higher than the rate estimates, that can be derived by the more indirect methods of using concentration gradients or ground water ages in deeper aquifer systems (Chapelle & McMahon, 1991; McMahon & Chapelle, 1991; Aravena et al. 1995). Overall, there is little doubt, that these differences mainly reflects the

availability of reactive organic matter (Berner, 1985). In aquifer systems the most important control on the rates is the age and reactivity of the organic matter present (Jakobsen & Postma, 1994). In the young and shallow Rømø aquifer, the organic matter is young and reactive, whereas the organic matter in deep aquifer systems is much older and accordingly has a low reactivity, since the most reactive part was decomposed long ago (Jakobsen & Postma, 1994). In marine and lake sediments the rates are influenced mainly by the sedimentation rate of organic matter, since all organic matter here is young, at least until it is buried deep into the sediments. As an example, the much studied marine Cape Lookout Bight has a very high rate of organic matter sedimentation and accordingly higher rates of sulfate reduction and methane production than many other marine sediments (Hoehler, 1998).

Cape Lookout Bight is the only one of the sites shown in table 5.1, where the maximum rate of sulfate reduction is considerably larger (six times) than the maximum rate of methane production. Most likely this reflects, that sulfate reduction takes place in the upper part of the sediment, where the most reactive organic matter is present. In lake sediments the penetration of sulfate into the sediment by diffusion and bioturbation is much smaller than in marine sediments, and accordingly the maximum rate of methane production might actually be higher in lake sediments than the maximum rate of sulfate reduction (Lake Washington). The values shown in table 5.1 for the Rømø aquifer are measured in the same sediment (location 3) at times where sulfate was present/absent (figure 4.8). The very similar values indicate, that it does not have any major impact on the rate of organic matter decomposition whether the TEAP is sulfate reduction or methane production. Perhaps because the energy released by these two processes is not very different, as shown in figure 1.1. Introduction of an electron acceptor with a much higher energy yield per mole carbon, like O_2 or NO_3^-, might lead to a different result, and it cannot be induced from these data, that the rate of organic matter decomposition is generally independent of the TEAP.

In marine and lake sediments, a rate maximum is usually found near the surface of the sediment and below this point the rate decreases with depth, due to a reduced availability of reactive organic matter (see e.g. some of the studies referred in table 5.1). In an aquifer, a similar distribution of rates over depth would be expected, if DOM (Dissolved Organic Matter), leaching from the soil zone, is the main electron donor for the redox processes, since the most reactive part of DOM would be expected to be decomposed first. The rate of acetate turnover (figure 4.17) decreases sharply with depth in the Rømø aquifer, and it is therefore likely, that DOM, leaching from the soil zone, is the main source of acetate. In principle, this should result in a decrease in DOC (Dissolved Organic Carbon) over depth in the upper part of the aquifer, but such a decrease would be very difficult to detect because of the large and unsystematic fluctuations in DOC near the groundwater table (figure 4.13).

However, the highest rates of methane production (this study) and sulfate reduction (Jakobsen, 1995) are measured in the deeper part of the aquifer, which speaks against DOM as the only electron donor. It might be argued of course, that the total rate of organic matter decomposition could still decrease with depth, if other redox processes like Fe(III)-oxide reduction occur at high rates in the upper part of the aquifer. However, the rates of Fe(III)-oxide reduction, that can be calculated from concentration-depth profiles (figure 4.5 and Jakobsen, 1995), result in carbon oxidation rates, that are considerably lower than those resulting from sulfate reduction and methane production. Accordingly the carbon oxidation rates are actually higher in the deeper part of the aquifer at many of the locations, which speaks against DOM as the main electron donor.

Moreover, a significant decrease in DOC with depth should be detected, if DOM was the main electron donor. Judging from the measured concentrations of SO_4^{2-} and CH_4 (figure 4.5), approximately 0.3 mM sulfate is being reduced and 0.3 mM methane produced on average over the sampled depth interval. Assuming an average oxidation state of zero for organic carbon, these two redox processes alone requires the oxidation of approximately 1.2 mM carbon. This is similar to the amount of DOC measured in most samples, and it is therefore highly unlikely, that a decrease in DOC with depth would remain undetected (as it is, according to figure 4.13 and Jakobsen, 1995) in so many concentration-depth profiles, if DOM was the only electron donor for the redox processes.

To illustrate the distribution of organic matter decomposition over depth, the carbon oxidation rates were approximated from the measured acetate turnover rates (both acetate fermentation and acetate oxidation) and CO_2 reduction rates. This approximation includes the contribution to carbon oxidation from acetate, that is utilized by other than methanogenic bacteria, and should therefore be very close to the actual carbon oxidation rates. In the sulfate containing parts of the aquifer, the measured sulfate reduction rates were used to approximate the carbon oxidation rates. This approximation might seriously underestimate the rate of carbon oxidation, when the sulfate reduction rate is low, since reduction of e.g. Fe(III)-oxides is likely to be quantitatively important in this situation. However, the approximation should be sufficiently close to provide an overview of where organic matter decomposition occurs at high/low rates. The results are shown in figure 5.1. Also shown in figure 5.1 is the concentrations of Ca^{2+} at the various locations, to illustrate where Ca-carbonate buffering takes place (the implication of this will be discussed later).

There are two zones in the aquifer, where high carbon oxidation rates are found. One is found at shallow depths (above 2 m.b.s.), and the other is found in a zone of 1-2.5 m after the Ca^{2+} concentration has increased due to Ca-carbonate dissolution. The high carbon oxidation rates in the upper part of the aquifer are mainly due to turnover of acetate, and, as previously suggested, it could be DOM, leaching from the soil zone, that is being decomposed here. The high rates in the upper

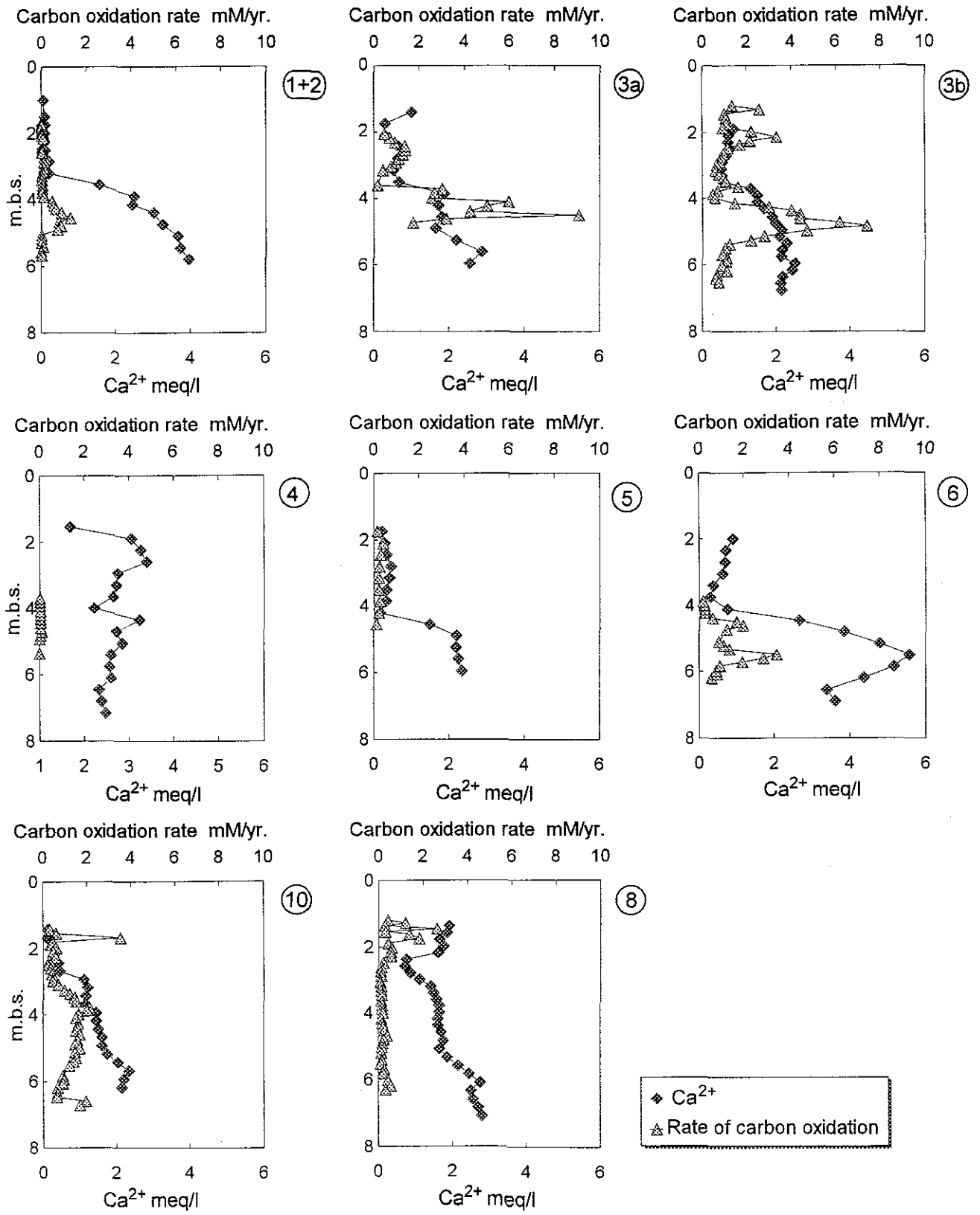

Figure 5.1. Approximated total rates of carbon oxidation and measured Ca^{2+} concentrations in the Rømø aquifer. Data from location 1+2,4, 5 and 6 are from (Jakobsen, 1995), data from location 10 and 8 are from this study. The data from location 3a and 3b are two sets of measurements from the same location, carried out by (Jakobsen, 1995) (a) and by this author (b).

part of the Ca-carbonate containing zone are more puzzling. Apparently the increase in Ca^{2+} concentration and/or the increase in pH, that results from Ca-carbonate dissolution, has a positive effect on the rates of organic matter decomposition. Alternatively, reactive organic matter from carbonate shells are being decomposed, when these dissolve.

To test this hypothesis, and to test, if SOM (Sedimentary Organic Matter) in general is a realistic electron donor for the redox processes, it was calculated, how many years it would take to oxidize the SOM present in the sediment at location 3, if SOM was the only electron donor. The results are shown in figure 5.2.

If SOM was the only electron donor for the redox processes at location 3, it would be used up within 10-100 years at most depths at the rates measured in July 1997. Assuming a SOM content at the other locations similar to location 3, the turnover times of SOM here would approximately be 15-400 years at the rates measured in July 1997. These values, or at least the lower end of the range, seem unrealistically low, since the sedimentary HA`s has an age of 500-2400 years (table 4.1). Moreover, it was shown in section 4.3, that a fraction of the SOM is young organic matter, leached by infiltrating water from the present soil zone. If old SOM was the only electron donor, it would therefore have to be decomposed even faster than calculated here. Only at the depth (6,2 m.b.s.) at location 3, where 10-20 times more SOM is present, do we find somewhat realistic, albeit still rather low, turnover times of SOM of up to 1000 years.

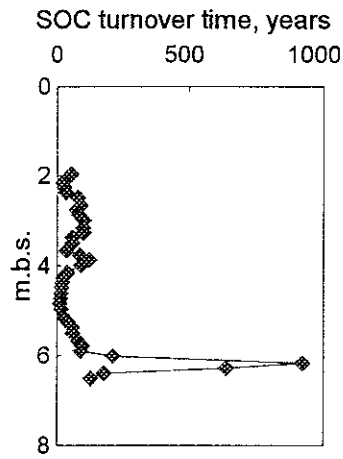

Figure 5.2. Turnover time of SOC at location 3, if SOC was the only electron donor for the redox processes

Thus old SOM (whether related to shells or not) cannot be the main electron donor for the redox processes in the deeper part of the aquifer. This is further supported by the very poor correlation between rates and SOC (Sedimentary Organic Carbon) at location 3. The highest redox rates at location 3 are not measured at 6,2 m.b.s., where a lot of SOC is present (figure 4.14), but at other, more shallow depths (figure 4.16). On the other hand, a small peak in the redox rates is actually found at location 3, 6,2 m.b.s. And high rates were also measured in a few of the deepest samples from location 10 (7 m.b.s.), where the sediment had a similar visual appearance. Accordingly, the old SOM is being slowly decomposed, but this process is quantitatively important only in local layers with a high content of SOM in the marine part of the sediment.

What then is the main source of the organic matter, that is being decomposed in the deeper part

of the aquifer? The most plausible explanation is, that a significant fraction of the mobile organic carbon is not detected by the analytical procedure used for DOC. DOC is arbitrarily defined as the fraction, that passes through a certain filter size, in this study 0.2 µm cellulose acetate or cellulose nitrate membrane filters. But it has been reported, that a significant part of the organic matter present in reduced aquifers can be associated with mobile colloids (Ryan & Gschwend, 1990). Presumably in the form of organic coatings on small particles of clay. Such colloids might be removed by a filtration, particularly as the filters clog and the effective pore size is reduced (Danielson, 1982).

The hypothesis about colloidal transport of organic matter was developed relatively late in the investigation, and no measurements of the amount of organic carbon associated with colloids are available. Therefore there is no direct evidence to support the hypothesis, but it is consistent with the observations, that were made during field work. In the upper, calcite free part of the aquifer, clogging of filters occurred very fast during filtration. In contrast, much larger amounts of water from the deeper, calcite containing parts of the aquifer, could be filtered, before clogging of filters occurred. This indicates, that more colloids are present in the upper part of the aquifer, and that these are immobilized, when the water enters calcite containing parts of the aquifer. These observations are consistent with the findings of others. It is well known that a coating with organic matter stabilises colloids and that high concentrations of Ca^{2+} destabilize them (Stumm & Morgan, 1996). Also the increase in pH caused by calcite dissolution may result in destabilisation of colloids (Stumm & Morgan, 1996).

The content of SOM in the upper part of the calcite containing zone (figure 4.14) is not significantly higher than elsewhere in the sediment. Accordingly, if colloidal organic matter is deposited here, it must be decomposed again within a few years. The high rates of carbon oxidation in these parts of the aquifer (figure 5.1) supports, that this occurs. The fact (section 4.3), that a part of the SOM (fulvic acids) is very young, is also in agreement with the proposed model.

Moreover, the large differences in rates, that are found between the sampling locations, are also readily explained by the proposed model. The highest rates were measured at location 3, which is situated very closely downstream a large winter wet pond (figure 3.2). Possibly, this leads to a higher content of (colloidal) organic matter in the water found at location 3, because the water has passed through only a short unsaturated zone or none at all. In support of this, it was observed in the field, that filtration in the upper part of the aquifer at location 3 was more difficult than anywhere else. The infiltration of water from the winter wet pond also increases the flow velocity in nearby downstream parts of the aquifer (figure 3.2). This could also increase the rate of carbon oxidation, because more water is transported through a certain volume of sediment in a given time. The aquifer then acts as a kind of filter: The higher flow velocity through the filter, the more

organic matter is collected on the sediment in a given time.

The influence of the winter wet pond might also explain, why the CO_2 reduction rates were so much lower at location 3 in October 1996 (figure 4.16). At this time, the pond had been dried out for several months. This has two effects: The flow rate was lower in October 1996 than in July 1997 or April 1993 (when the pond was wet), and the water came from another direction because the pond did not influence the flow direction (figure 3.2). According to the proposed model, this should result in a decreased rate of carbon oxidation. The water found at location 10 below 3 m.b.s. has also infiltrated in areas with a very shallow water table (although not winter wet), which might explain the relatively high rates measured also at this location. When the water reaches location 8, 13 m downstream of location 10, most of the reactive organic matter must have been used up, since much lower rates are found there.

In conclusion, most of the organic matter being decomposed in the Rømø aquifer must be leached from the soil zone by infiltrating water and colloidal transport of organic matter seems to control the distribution of high/low rates in the aquifer. A similar situation seems to exist in many other aquifers with a shallow water table. (Starr, 1988) reported that the organic matter responsible for nitrate reduction in a shallow aquifer come from the soil zone and that nitrate reduction does not take place in another aquifer with a thicker (5 m) unsaturated zone. This has the important implication, that a lowering of the groundwater table is likely to reduce the input of reactive organic matter to shallow aquifers and thereby diminish their ability to reduce nitrate and sulfate in the infiltrating water.

The apparent influence of colloidal transport is not limited to the Rømø aquifer either. (Johansen, 1997) measured sulfate reduction rates and methane production rates in the shallow Asserbo aquifer and found a distribution of rates over depth, that is very similar to the one found in the Rømø aquifer (the magnitude of rates is also similar). Moreover, mass balance calculations, as those carried out for the Rømø aquifer in this section, show, that SOM or DOM cannot be the main electron donor in the Asserbo aquifer either. Accordingly, colloidal transport of organic matter seems to be important in the Asserbo aquifer as well.

5.2 Segregation of redox processes

The in situ rate measurements (figure 4.15 and 4.16) and the concentration profiles of SO_4^{2-} and CH_4 (figure 4.5 and 4.11) all show, that sulfate reduction and methane production are spatially separated in the Rømø aquifer. Moreover, the changes in water chemistry, that occurred at some of the sampled locations between 1992/1993 and 1995/1997 (figure 4.8) show, that the introduction of sulfate into previously methane containing parts of the aquifer inhibits methanogenesis, while methane readily forms in previously sulfate reducing parts of the aquifer, when sulfate

is no longer present. On the other hand, methane production was never completely excluded in any of the samples analysed, and some of the concentration depth profiles of sulfate and methane from the Rømø aquifer measured by (Jakobsen & Postma, 1994; Jakobsen, 1995) show large overlaps between methane and sulfate containing water. In conclusion the bulk of evidence indicates a distinct, but not complete, separation between sulfate reduction and methane production in the Rømø aquifer.

This observation is in accordance with the theory about competitive exclusion (Abram & Nedwell, 1978; Lovley et al. 1982; Lovley & Klug, 1983) and with many other field studies from both aquifers and other sediments (e.g. Sansone & Martens, 1982; Zhang et al. 1998). However, some studies (e.g. Senior et al. 1982; Crill & Martens, 1986), have demonstrated concurrent sulfate reduction and methane production in sediments, so a distinct separation between sulfate reduction and methane production is not universally found.

In contrast, there is no distinct separation between Fe(III)-oxide reduction and sulfate reduction. At all the locations sampled in this work as well as at some of those sampled by (Jakobsen, 1995), the sulfate reduction rates (figure 4.15) and concentration-depth profiles of Fe^{2+} (figure 4.5) indicate concurrent reduction of sulfate and Fe(III)-oxides. Concurrent reduction of sulfate and Fe(III)-oxides has also been reported to occur in marine sediments (Canfield et al. 1993). As mentioned in section 1.1, this lack of separation was proposed by (Postma & Jakobsen, 1996) to be the result of the thermodynamic feasibility of concurrent reduction of Fe(III)-oxides and sulfate.

Whether methane production and Fe(III)-oxide reduction are spatially separated in the Rømø aquifer is less clear. Increasing Fe^{2+} concentrations are generally not found in the methane containing parts of the aquifer. On the other hand, reducible Fe(III)-oxides are present at all depths (Jakobsen, 1995; Larsen, 1998), and the occurrence of methane production is therefore not due to a lack of reducible Fe(III)-oxides. Also the water is often supersaturated 5-15 times with siderite (figure 4.6), and siderite precipitation, along with ion exchange reactions and sorbtion of Fe^{2+} to organic matter, might prevent the detection of Fe(III)-oxide reduction from the concentration depth profiles.

The measured rates of methane production (figure 4.16) show, that another redox process is outcompeting methane production in sulfate free parts of the aquifer (2-3 m.b.s. at location 10 and 2-4 m.b.s. at location 8). The rate measurements carried out with 2-^{14}C-acetate (figure 4.17) also shows, that a significant fraction of the methyl group of acetate ends up as $^{14}CO_2$, even in the methanogenic parts of the aquifer, which indicate the concurrent occurrence of another redox process. This redox process could be Fe(III)-oxide reduction. However, assuming a vertical flow velocity of 1.25 m/yr. (section 3) and horizontally homogeneous rates, there are parts of the

aquifer, where the measured acetate oxidation rates would result in the formation of much larger amounts of Fe^{2+} than actually are found in the water, if Fe(III) was the only electron acceptor for acetate oxidation. Moreover, this is true even when the possible precipitation of siderite and iron sulfides is considered. The acetate oxidation rates would also result in a very rapid depletion of the sedimentary pool of Fe(III)-oxides (2-40 years at location 3) below the sulfate reducing zone, if sedimentary Fe(III)-oxides were the sole electron acceptor for acetate oxidation.

On the other hand the availability of other electron acceptors is sparse. Oxygen, nitrate, and sulfate are absent and Mn(IV)-oxides are of minor importance only (figure 4.5). One of the few remaining possibilities is the reduction of organic matter to other substances than CH_4. Such processes might occur, but there is no evidence to support it. Alternatively the apparent oxidation of acetate could be due to isotope exchange of the methyl carbon, but to my knowledge this has not been reported to occur. And isotope exchange could still not explain the suppression of methane production occurring in some sulfate free parts of the aquifer as Fe(III)-oxide reduction could.

Finally, the oxidation of the ^{14}C-labelled methyl group in 2-^{14}C-acetate could be carried out by methanogenic bacteria, that turns the unlabelled carboxyl group into methane instead of the labelled methyl group. Methanogenic bacteria exists, that are able to do this. (Krzycki et al., 1982, referred by Vogels et al., 1988) reported, that this pathway of methane production accounted for 15 % of the methane produced by an acetate-adapted strain of *M. Barkeri*. However, in the Rømø aquifer, up to 99 % of the methyl group of acetate is oxidized to CO_2 and the RI varies highly in the sulfate free parts of the aquifer. This strongly indicates, that it cannot be methanogenesis by this unusual pathway, that are responsible for the oxidation of the methyl group of acetate. Their must be another competing redox process, e.g. Fe(III)-oxide reduction.

Theoretically there is no reason, why the reduction of Fe(III)-oxides should not take place concurrently with the production of methane. Just as is the case for concurrent reduction of sulfate and Fe(III)-oxides (Postma & Jakobsen, 1996), the process is thermodynamically feasible under a wide range of natural conditions. However, slightly more stable Fe(III)-oxides are necessary for equilibrium between Fe(III)-oxide reduction and methane production, because methane production is energetically less favourable than sulfate reduction. Accordingly, there could be situations, where Fe(III)-oxide reduction can outcompete methane production but not sulfate reduction. Perhaps this is the reason, why no methane was found below the sulfate containing zone at location 1 in August 1995 (figure 4.8).

To examine the relation between in situ Gibbs free energy, ΔG_r, of different TEAP`s and the segregation between TEAP`s, ΔG_r`s for the various redox processes were calculated from the equations and thermodynamic values shown in table 5.2. Corrections for temperature were made

using the reaction enthalpy and the Van't Hoff equation in the form:

$$\Delta G^{\circ T_2} = \frac{\Delta H^{\circ}(T_1 - T_2) + T_2 \Delta G^{\circ T_1}}{T_1}$$

where ΔH° is the standard state reaction enthalpy, T_1 is the absolute temperature at standard conditions (298.15 K) and T_2 is the absolute groundwater temperature (281.15 K).

TEAP	Equation used for calculating in situ ΔG_r	$\Delta G^{281.15}$ (kJ/mole)	ΔH_r^0 (kJ/mole)
$H_2 + 2FeOOH + 4H^+ \rightarrow 2Fe^{2+} + 4H_2O$	$\Delta G_r = \Delta G^T + RT\ln\frac{[Fe^{2+}]^2}{[H_2][H^+]^4}$	-178,4 (I) -157,5 (II)	-198,7
$4H_2 + SO_4^{2-} + H^+ \rightarrow HS^- + 4H_2O$	$\Delta G_r = \Delta G^T + RT\ln\frac{[HS^-]}{[H_2]^4[SO_4^{2-}][H^+]}$	-262,3	-235,0
$HCO_3^- + 4H_2 + H^+ \rightarrow CH_4 + 3H_2O$	$\Delta G_r = \Delta G^T + RT\ln\frac{[CH_4]}{[H_2]^4[H^+][HCO_3^-]}$	-229,4	-237,8
$CH_3COO^- + 8FeOOH + 15H^+ \rightarrow 8Fe^{2+} + 2HCO_3^- + 12H_2O$	$\Delta G_r = \Delta G^T + RT\ln\frac{[Fe^{2+}]^8[HCO_3^-]}{[CH_3COO^-][H^-]^{15}}$	-499,0 (I) -415,4 (II)	-566,3
$CH_3COO^- + SO_4^{2-} \rightarrow HS^- + 2HCO_3^-$	$\Delta G_r = \Delta G^T + RT\ln\frac{[HS^-][HCO_3^-]^2}{[CH_3COO^-][SO_4^{2-}]}$	-47,6	-6,4
$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^-$	$\Delta G_r = \Delta G^T + RT\ln\frac{[CH_4][HCO_3^-]}{[CH_3COO^-]}$	-14,7	-9,2
$CH_3COO^- + 4H_2O \rightarrow 2HCO_3^- + H^+ + 4H_2$	$\Delta G_r = \Delta G^T + RT\ln\frac{[HCO_3^-]^2[H^+][H_2]^4}{[CH_3COO^-]}$	214,7	228,6

Table 5.2 Equations and thermodynamic values used for calculating in-situ Gibbs free energies for terminal electron acceptor processes (TEAP's) in the Rømø aquifer. R is the gas constant and T is the absolute temperature in K, [ ] indicate species activities.

The thermodynamic data on solutes used in the calculations, are from (Stumm & Morgan, 1996). The ΔG_r^0 values for Fe-oxide reduction corresponds to the reduction of the least (ΔG_f^0=-472.8 kJ/mole) and most (ΔG_f^0=-483.3 kJ/mole) stable lepidocrocites (Langmuir, 1997). The Fe(III)-oxides present in the Rømø aquifer have reactivities covering the entire span from ferrihydrite to goethite (Larsen, 1998), and lepidocrocite was arbitrarily chosen as an example. The ΔH_r^0 values for the lepidocrocite reactions were calculated using the ΔH_f^0 = -559.4 kJ/mole for goethite since no value is available for lepidocrocite. The error introduced by this approximation is minor

because the effect of ΔH is small for small temperature differences, and also the temperature effect on ΔG^T is small. The main temperature effect comes from the second part of the equation.

The calculations of in situ Gibbs free energies were performed using the concentrations of Fe^{2+}, CH_4 and SO_4^{2-} measured in July 1997. The HCO_3^- activities were calculated by PHREEQC (Parkhurst & Appelo, 1997) from TIC and pH values measured in July 1997. The H_2 concentrations measured in March 97 were used for location 8 and 10. For location 3 the average of the H_2 concentrations measured in March and July 1997 were used. The acetate concentrations measured in July 1997 were used for location 8 and 10 and the average of the concentrations measured in 1996 for location 3. As for the calculation of acetate turnover rates (section 2.5.3), narrow peaks in the acetate concentration were neglected. Activity and complexion corrections were made by entering the molar concentrations in PHREEQC (Parkhurst & Appelo, 1997), using the PHREEQC database with values for acetate complexion added from the MINTEQ database (Westall, Zachary & Morel, 1976). The concentrations of cations other than Fe^{2+} used in PHREEQC (Parkhurst & Appelo, 1997) calculations were those measured in 1996. The concentrations of these cations are generally quite constant in the Rømø aquifer and they have only influence on the complexion and activity corrections. Cl^- was adjusted to achieve electroneutrality.

The concentrations of S(II) were not measured, since they are at or below the detection limit of 1 µM (Jakobsen, 1995). Accordingly the concentration of S(II) was set to 1 µM in all samples and the concentration of HS^- was calculated with PHREEQC (Parkhurst & Appelo, 1997). If the actual concentrations of S(II) were lower than 1 µM, then the ΔG_r for sulfate reduction is slightly more negative (higher energy yield) than calculated here. However, since the concentration of HS^- only enters into the logarithmic equations once, very large changes in the HS^- concentration are necessary to seriously affect ΔG_r for sulfate reduction. Even a 100 fold decrease in the HS^- concentration would only increase the energy available for sulfate reducers by ~2.7 kJ/mole H_2 and ~10.8 kJ/mole acetate, which is minor compared to the differences between the various TEAP`s under most conditions. The calculated in situ Gibbs free energies are shown in figure 5.3.

The suppression of CO_2 reduction by the presence of sulfate is consistent with the in situ ΔG_r values shown in figure 5.3. In all the samples, where sulfate is present, the ΔG_r for CO_2 reduction is zero or positive, and CO_2 reduction can therefore not occur at the H_2 concentrations measured in the sulfate reducing zone. It is more difficult to explain, why methane production from acetate is being suppressed. Sulfate reduction has a much larger energy yield per mole acetate than methane production (~70 kJ/mole acetate and ~30 kJ/mole acetate respectively). However, the ΔG_r for acetate fermentation is always negative (-15 to -30 kJ/mole acetate) and appears to be completely unrelated to where the process occurs (figure 4.16). The highest energy yield is actually found in the sulfate reducing zone, where the bulk of the acetate is turned over oxidatively

perhaps close to the limit

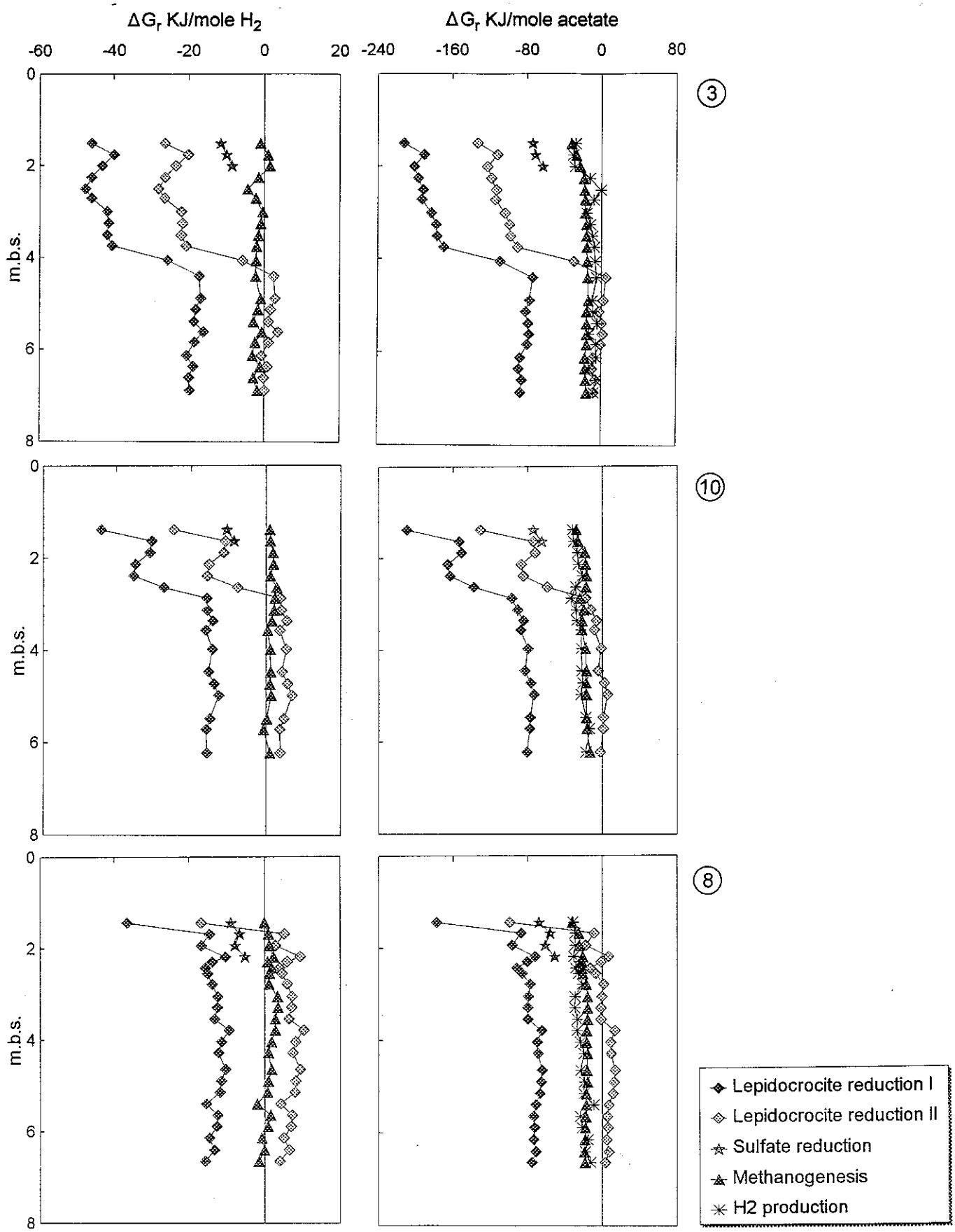

Figure 5.3. In situ Gibbs free energy, ΔG_r, for lepidocrocite reduction (least and most stable forms), sulfate reduction and methane production from H_2 and from acetate at three locations (encircled numbers) in the Rømø aquifer. Also shown is ΔG_r for H_2 production from acetate. Negative ΔG_r implies a positive energy yield of the reaction. Details of the calculations are found in the text.

(figure 4.17). This is somewhat puzzling, since the whole concept of competitive exclusion of redox processes is based on the ability of the more favourable TEAP to keep the concentration of e.g. hydrogen or acetate down to a level, where the energy yield of other, less favourable, TEAP`s becomes too low for these processes to occur (Lovley et al. 1982; Chapelle & Lovley, 1992; Lovley & Phillips, 1987).

The occurrence of CO_2 reduction is not easily explained from the calculated ΔG_r values (figure 5.3) either. While the ΔG_r values for CO_2 reduction are generally slightly more negative in the parts of the aquifer, where the process takes place at appreciable rates (below 2 m.b.s. at location 3, below 3 m.b.s. at location 10 and below 4 m.b.s. at location 8), there are no parts of the aquifer, where they are < -4 kJ/mole H_2. While it can perhaps not be ruled out completely, that CO_2 reduction could occur at such a low ΔG_r, much higher threshold levels have been found by others. (Schulz & Conrad, 1996) reported a threshold level for CO_2 reduction (at 4 °C) of -7 kJ/mole H_2, (Hoehler, 1998) reported a threshold level of -10 to -15 kJ/mole H_2 in Cape Lookout Bight sediment.

For the reduction of Fe(III)-oxides, an inverse relationship is found between the site, where Fe(III)-oxide reduction is most energetically favourable (location 3) and the site where the highest concentrations of Fe^{2+} are found (location 8) in the upper part of the aquifer (figures 5.3 and 4.5). Since the rates of sulfate reduction and methane production are much higher at location 3 than at location 8 (figure 4.15 and 4.16), this cannot be due to a rate limiting availability of reactive organic matter at location 3. Rather the Fe(III)-oxides present at location 8 must be more reactive than those present at location 3. In conclusion it is not possible to obtain useful information from the calculated ΔG_r values for lepidocrocite reduction, since the stability of the Fe(III)-oxides present is unknown and apparently varies a lot between the different sampling locations. Apart from mineral stability, the most important factor controlling ΔG_r for Fe(III)-oxide reduction in the Rømø aquifer is the pH, as indicated by the large increase in ΔG_r for lepidocrocite reduction occurring around 4 m.b.s. at location 3 and around 3 m.b.s. at location 10. The concentration of Fe^{2+} also has a large effect, as indicated by the high ΔG_r values for lepidocrocite reduction found at all but the shallowest depth at location 8.

5.3 Dynamics of H_2 and acetate and the significance of their concentration levels

The lack of correlation between the measured in situ rates of redox processes (figure 4.15, 4.16 and 4.17) and their energy yields (figure 5.3), raise several questions about the cycling of H_2 and acetate in sediments and the significance of their concentration levels.

The very low energy yield for CO_2 reduction, even in parts of the aquifer where the rate measurements indicate appreciable rates, strongly suggests, that a large part of the CO_2 reduction

takes place within micro niches containing much higher H_2 concentrations or by interspecies hydrogen transfer between juxtaposed bacteria, as proposed by (Conrad et al. 1985; Thiele & Zeikus, 1988). The juxtaposition arrangement might be thought of as a micro environment with a higher H_2 concentration between the cell walls of two bacteria. Accordingly, the two phenomenons have the same effects, and, for the sake of simplicity, the term micro niches will be used in the following to describe them both .

Since the energy yield for CO_2 reduction at the bulk water H_2 concentration is too low for the process to occur, another TEAP, e.g. Fe(III)-oxide reduction, must control the bulk water H_2 concentration in the Rømø aquifer, even when methane production actually is the dominating TEAP. This seriously invalidates the whole concept of using H_2 concentrations as indicator for the TEAP as proposed by (Lovley & Goodwin, 1988). (Lovley & Goodwin, 1988) actually discussed the possible influence that micro niches might have on the concept, but concluded, that the H_2 concentration in the bulk sediment would be unaffected, as long as just a minor fraction of the H_2 is turned over there. This is theoretically correct, but only if one TEAP is active at a time, which is not always the case (e.g. Canfield et al. 1993; Postma & Jakobsen, 1996, this study).

In conclusion, the concentration of hydrogen might still be used as an indicator of the dominant TEAP, but the obtained results will only be reliable in sediments, where one TEAP dominates completely. Moreover, recent studies (Hoehler, 1998) have shown, that the H_2 concentration in sediments depends not only on the TEAP, but also on other factors affecting the ΔG_r values (temperature and the concentrations of the involved species), as would be expected, if the bacteria keeps the H_2 concentration as close to thermodynamic equilibrium as biologically possible. Rather than using empirically defined concentration ranges, as those proposed by (Lovley & Goodwin, 1988; Chapelle et al. 1995), the actual in situ ΔG_r values for the different TEAP`s should be calculated and compared to the expected threshold levels for the TEAP`s. Still, where possible, the diagnosis of TEAP is best made by measuring the rates directly.

If CO_2 reduction in the Rømø aquifer occurs within micro niches, one might ask, why the process does not take place at appreciable rates everywhere in the aquifer? A possible answer is, that other bacteria, e.g. Fe(III)-oxide reducing and sulfate reducing, are able to outcompete the methanogens for H_2 inside the micro niches in some parts of the aquifer, but not in others. For sulfate reducers this is easy to understand. Sulfate is present in millimolar concentrations and diffusion will therefore rapidly replace any sulfate, that might be reduced within micro niches. Accordingly, the only competitive advantage that methanogens could have over sulfate reducers, even in micro niches, would be due to a size exclusion of the rather large sulfate reducing bacteria (cell lengths of 0.9-11 µm according to (Widdel, 1988)). (McMahon & Chapelle, 1991) proposed, that sulfate reducers were excluded from aquitards because of the small pore size, whereas fermenting bacteria

were not. However, to my knowledge, a similar phenomenon has not been reported to be important in the competition between methanogens and sulfate reducers.

In some sulfate free parts of the aquifer, another redox process is apparently also able to prevent CO_2 reduction within micro niches. This redox process could be Fe(III)-oxide reduction, but this requires, that all micro niches contain Fe(III)-oxides in these parts of the aquifer. The occurrence of CO_2 reduction in other parts of the aquifer (within micro niches) must then be due to a rate limiting low reactivity of the Fe(III)-oxides present there and/or a more uneven distribution of them.

Alternatively, the measured H_2 concentrations are significantly lower than the actual concentrations, but there is no evidence to support this. (Frank, 1996) measured H_2 concentrations at location 3, using a completely different method (collection of gas from the headspace of sealed micro-wells, installed in the aquifer) and found concentrations similar to those measured in this study by the bubble stripping method (section 2.4).

The ΔG_r values for sulfate reduction from H_2 are also quite low (-7 to -10 kJ/mole H_2, figure 5.3). This could indicate, that sulfate reduction from H_2 also takes place within micro niches. The concentration of hydrogen would then be controlled by another redox process, e.g. Fe(III)-oxide reduction, also in the sulfate containing parts of the aquifer. In support of this, the concentration of hydrogen does not increase significantly at the depth of sulfate depletion at location 10 and 8, whereas (Jakobsen, 1995) measured higher H_2 concentrations (about 1 nM rather than 0.2-0.4 nM) in some sulfate containing parts of the aquifer than those found in this study . However, threshold values for CO_2 reduction as low as 7 kJ/mole H_2 at 4 °C have been reported (Schulz & Conrad). If sulfate reduction from H_2 can occur at a similar low energy yield, the H_2 concentration could be controlled by sulfate reducers in the sulfate containing parts of the aquifer.

For all TEAP`s, the energy yield is appreciably lower for the reaction involving acetate than for the reaction involving H_2 (figure 5.3). This is true when the comparison is made on a mole substrate basis, as in figure 5.3, but also when the comparison is made on a mole electron acceptor basis. The in situ ΔG_r value for oxidation of acetate to $CO_2 + H_2$ (figure 5.3) is negative in all but a single sample, which means that oxidation of acetate to $CO_2 + H_2$ is always energetically favourable. Accordingly, all the TEAP`s has a larger energy yield per mole electron acceptor from acetate than from H_2. Either way we may look at it, the redox processes involving acetate are further from thermodynamic equilibrium than those involving H_2. This indicates, that the acetate utilizing bacteria requires a larger energy yield than the hydrogen utilizing bacteria. The reason for this is perhaps, that acetate is a much larger and more complex molecule than H_2. It is also evident, that methanogenic bacteria can keep the concentration of acetate much closer to thermodynamic

equilibrium than the sulfate reducing bacteria, since the energy yield for sulfate reduction from acetate is more than 3 times higher than the energy yield at which acetate fermentation takes place (figure 5.3 and 4.16).

As shown in the previous section, the suppression of methane production from acetate in parts of the aquifer is not due to a low acetate concentration or to a low energy yield of the process . Other studies have however shown lower acetate concentrations in sulfate reducing than in methane producing parts of a sediment. (Kuivila et al. 1988) found that the acetate concentration was 10-15 µM in the sulfate reducing part of Lake Washington sediment, and 30-40 µM in the methanogenic part of the sediment. Lower acetate concentrations have also been reported in Fe(III)-oxide reducing parts of an aquifer (0.5-2.0 µM) than in sulfate reducing parts of the aquifer (2.0-3.0 µM) (Chapelle & Lovley, 1992).

The acetate concentration has, however, only a limited effect of the energy yield of the TEAP`s. The change in $\Delta G_r$ for sulfate reduction and methane production from acetate accomplished by the 2-4 fold change in the acetate concentration between different TEAP`s, that was found by (Kuivila et al. 1988; Chapelle & Lovley, 1992), is only a few kJ/mole acetate. While it perhaps cannot be ruled out completely, such a small change in ΔG_r seems unlikely to inhibit a redox process.

Possibly the methanogenic bacteria are not being outcompeted for acetate by sulfate reducers or Fe(III)-oxide reducers, but the low H_2 concentration maintained by these bacteria causes them to switch their metabolism from fermentative to oxidative cleavage of acetate, where the electrons are released in the form of H_2 rather than as CH_4. Studies in well defined cocultures of acetate consuming methanogens and H_2 consuming sulfate reducers have demonstrated the ability of certain strains of acetogenic methanogens to switch their metabolism from fermentative to oxidative cleavage of acetate, where the electrons are presumably released in the form of H_2 (Phelps et al. 1985). This switch took place, when the H_2 concentration was kept down by sulfate reducers.

There is also evidence for the occurrence of the process in natural environments. (Achtnich et al. 1995) investigated the turnover of acetate and H_2 in a methanogenic paddy soil and demonstrated, that addition of sulfate or Fe(III)-oxides to the sediment resulted in large decreases in the H_2 concentration while the acetate concentration remained constant, except from a temporary increase, when sulfate was added. Inhibition experiments with chloroform showed, that the sulfate reducers present in the sediment could not utilize acetate directly, and that methanogens switched at least partly from methane production to H_2 production from acetate, when sulfate or Fe(III)-oxides was added to the sediment.

In general there is a good agreement between the sites, where high H_2 concentrations were measured (figure 4.10) and the sites, where methane production from both H_2 and acetate occurs at appreciable rates (figure 4.16). The calculated in situ ΔG_r values (figure 5.3) show, that H_2/CO_2 production from acetate is more energetically favourable than CH_4 production from acetate in all parts of the aquifer, where low methane production rates and a high respiration index was measured (above 2 m.b.s. at location 3, above 3 m.b.s. at location 10 and above 4 m.b.s. at location 8). In contrast, the CH_4 production from acetate is generally more favourable, or the two processes are equally favourable, in the parts of the aquifer, where high methane production rates and a low respiration index was measured.

The ΔG_r values at location 10 deviates a bit from this general picture. The reason for this is not known, but it is possible, that the concentration of H_2 changed substantially here between March 1997, when the H_2 concentrations were measured and July 1997, when the rates were measured. The low concentrations of methane and the occasional presence of sulfate in this part of the aquifer (figure 4.5) indicate, that location 10 is situated very close to the point, where methane production replaces sulfate reduction along this flowline. Accordingly the H_2 concentration could fluctuate considerably here, depending on whether sulfate reduction or methane production is the main TEAP.

In conclusion, the H_2 concentration level could be the main control on methane production from acetate by causing a switch in the metabolism of acetate utilizing methanogens from the production of methane to the production of hydrogen, when the hydrogen concentration is low enough to make this energetically favourable. In the sulfate containing parts of the aquifer, the H_2 produced this way could be consumed by sulfate reducers and/or Fe(III)-oxide reducers, and in the parts of the aquifer that are free of sulfate, the Fe(III)-oxide reducers could consume the hydrogen. H_2 production from acetate could also explain the oxidation of acetate in methanogenic parts of the aquifer, but since it requires a low hydrogen concentration, the possible occurrence of this process does not remove the need of an electron acceptor other than CO_2 (methane production) in sulfate free parts of the aquifer. If most of the oxidative decomposition of acetate is shunted through hydrogen, an explanation is also provided for why sulfate reduction with acetate is as far as 70 kJ/mole acetate from thermodynamic equilibrium, since the energy yield would then have to be split between two bacteria: one that produces H_2 from acetate and one that consumes the H_2 (a sulfate reducer).

Alternative explanations for the high RI in parts of the aquifer are of course also possible. One such explanation could be, that the methanogenic bacteria are able to utilize acetate only by cometabolism with another substrate like hydrogen. The high respiration index in parts of the aquifer could then be explained by the outcompetition of methanogens for hydrogen (figure 4.16

and 4.17). However, this would be completely without precedence in the literature, at least to my knowledge. Another possibility could be the inhibition of acetate utilizing methanogens by a substance present in parts of the sediment. Inhibition of methane production from acetate by high concentrations of sulfide has been demonstrated (e.g. Gunnarsson & Rönnow, 1982), but in the Rømø aquifer this possibility can be excluded, because of the very low sulfide concentration. Finally the acetate utilizing methanogens could be inhibited by a lack of an essential nutrient like N or S, but it seems somewhat unlikely, that such a lack of nutrient would not affect the sulfate reducers and Fe(III)-oxide reducers as well.

While the differences between the acetate concentrations in zones with different TEAP`s are minor (Kuivila et al. 1988; Chapelle & Lovley, 1992) or absent (this study), the concentrations of acetate found in different sediments with the same TEAP varies up to four orders of magnitude (review of acetate concentrations in sulfate reducing marine sediments by (Sansone & Martens, 1982)). Also studies from polluted aquifers have shown concentrations of acetate and other fatty acids covering a span of several orders of magnitude, but with no correlation to the concentration of hydrogen (Vroblesky et al. 1997). In conclusion, no information, about which TEAP dominates, can be obtained from acetate concentrations.

It is interesting, that acetate concentrations can vary so much within the same TEAP, whereas the H_2 concentration is much more tightly controlled by the redox processes. At least two factors might be responsible for this. Firstly, the concentration of hydrogen is orders of magnitudes lower than the concentration of acetate (figure 4.10) and for this reason, the hydrogen pool is turned over much faster. To illustrate this point, the turnover times of H_2 and acetate in the Rømø aquifer were calculated from the rates measured in July 1997. The turnover of H_2 by other redox processes than methane production has not been measured directly. Since most of the methane production occurs via the CO_2 reduction pathway (figure 4.16), it was conservatively assumed, that the other redox processes occurred equally via H_2 and acetate, and the H_2 turnover time was approximated from the measured rates of CO_2 reduction and acetate oxidation. The results are shown in figure 5.4.

While the pool of H_2 is turned over in less than 1 minute in many parts of the Rømø aquifer, the pool of acetate is only turned over every 5-500 hours. This implies, that an increased production of hydrogen will very rapidly increase the concentration of hydrogen significantly and thus stimulate the growth of hydrogen utilizing bacteria. For acetate the link from production to consumption works much slower because of the larger pool size. Moreover, since 4 moles of H_2 but only one mole of acetate is needed for the reduction of one mole sulfate or production of one mole methane, a change in the H_2 concentration has a four times larger relative effect on the ΔG_r`s than does a similar change in the concentration of acetate. A 100 fold increase in the concentration

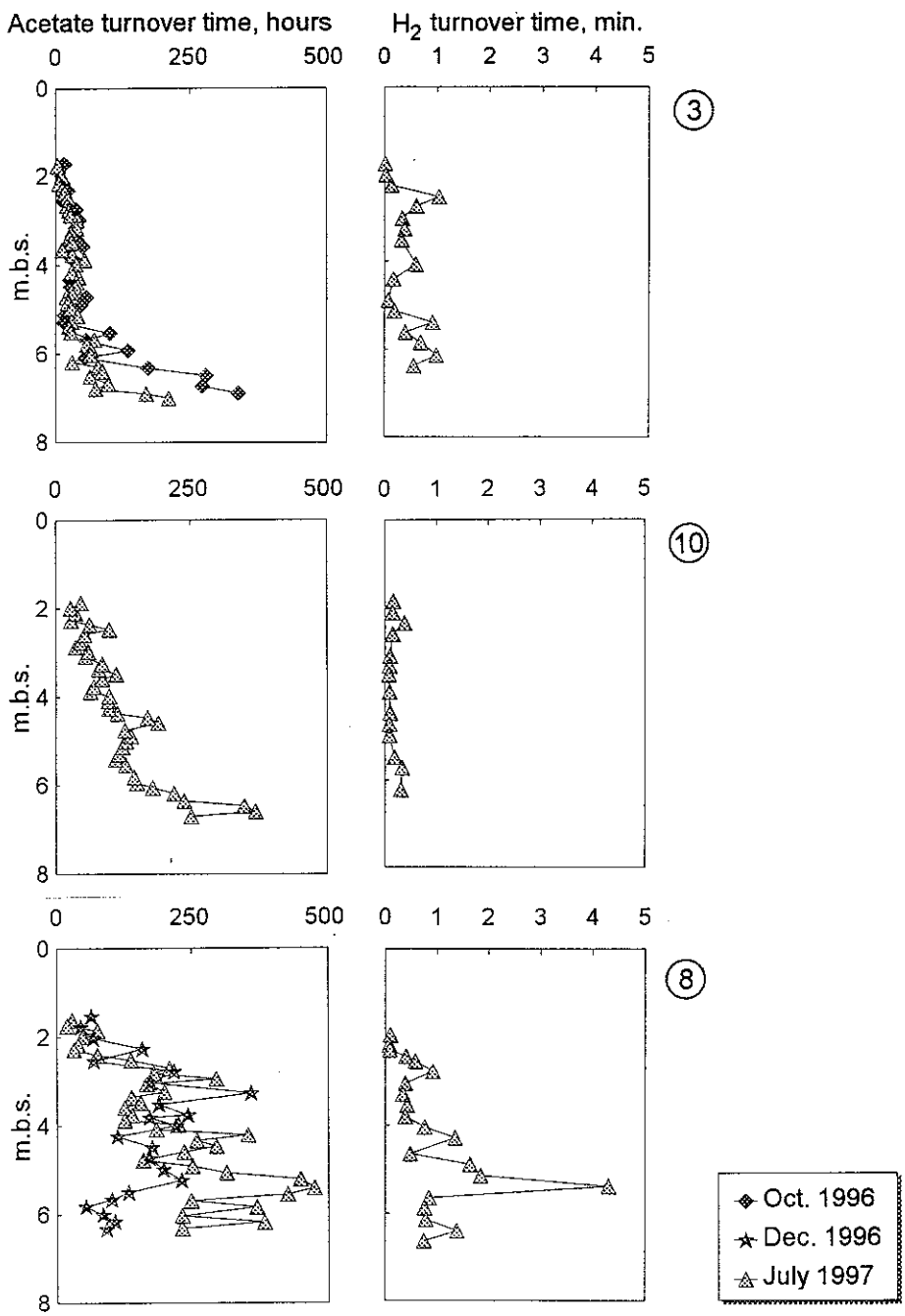

Figure 5.4. Approximated turnover times of H_2 and acetate at three sampling locations (encircled numbers) in the Rømø aquifer.

of acetate would for instance only lower ΔG_r for sulfate reduction from -70 to -80 kJ/mole acetate whereas a 100 fold increase in the concentration of hydrogen would lower ΔG_r for sulfate reduction from -10 to -20 kJ/mole H_2. This implies, that the link between production and consumption of acetate not only works slower, it is also likely to be much weaker, which might explain the findings of e.g. (Vroblesky et al., 1997).

The much larger turnover time of acetate also has the implication, that microniches with higher concentrations of acetate cannot form in homogeneous sediment at steady state. Diffusion and (in aquifers) advective transport will effectively level out any concentration differences, that might develop on a micro scale. On the other hand, higher acetate concentrations might be found in areas with a horizontal extension of centimetres to metres, if the redox processes are not at steady state. Possibly this is the reason for some of the local peaks and temporal variations in the acetate concentration in the Rømø aquifer (figure 4.8). In geologically diverse flow systems, large differences in the acetate concentration might also exist between the different types of sediment, as described by (McMahon & Chapelle, 1991).

To complete this minireview of acetate cycling in sediments, it should be mentioned, that a mechanism for acetate turnover in methanogenic sediments, that is almost opposite the one proposed here, has previously been proposed by (Sansone & Martens, 1982). In an attempt to explain the oxidation of acetate in sulfate free methanogenic sediment from Cape Lookout Bight, (Sansone & Martens, 1982) proposed, that acetate was oxidized to H_2 by sulfate reducers, when sulfate was absent and the H_2 subsequently used by methanogens. Since the CO_2 reduction rate is higher than the acetate oxidation rate in Cape Lookout Bight (Sansone & Martens, 1982; Crill & Martens, 1986) this is indeed a possibility here. However, (Sansone & Martens, 1982) used U-^{14}C-acetate, so perhaps some of the apparent acetate oxidation was actually isotope exchange (section 1.4).

Moreover, in the Rømø aquifer, the acetate oxidation rate is often much higher than the CO_2 reduction rate, which already excludes this explanation for the occurrence of acetate oxidation in sulfate free parts of the aquifer. Active sulfate reducers are however present below the depths of sulfate depletion, as indicated by the rapid turnover of injected $^{35}SO_4^{2-}$ in many samples with a low sulfate concentration. Moreover, the turnover of injected $^{35}SO_4^{2-}$ tracer was extremely high (up to 80 % within 24 hours) at the exact same site, where the highest acetate oxidation rates were measured. Therefore, a connection between acetate oxidation and sulfate reducers in the sulfate free part of the aquifer cannot be excluded. However, hydrogen produced from acetate by sulfate reducers could not be consumed by methanogenic bacteria, as suggested by (Sansone & Martens, 1982).

5.4 Controls on the methane concentration in deep aquifers

As discussed in the previous sections, the availability of reactive organic matter and the competition with other redox processes are the main factors controlling, whether methane is produced in appreciable amounts in aquifers. If the input of reactive organic matter from the soil zone is large, then methane might form shortly below the groundwater table in a shallow aquifer, but only after the oxygen, nitrate and sulfate present in the infiltrating water has been used up

(figure 4.5 and 4.8). In contrast, the presence of Fe(III)-oxides only prevents methane production, if the Fe(III)-oxides are reactive enough. The amount of methane, that forms in shallow aquifers is therefore mainly controlled by a mass balance between the amount of reactive organic carbon, leaching from the soil zone, and the amount of other, competing, electron acceptors, that are present in the infiltrating water.

In deeper aquifer systems, the same two factors - availability of reactive organic matter and competition with other TEAP´s - will also control the formation of bacterial methane. But in contrast to shallow unconfined aquifers, it is much more likely, that organic matter, deposited with the sediment, is the main electron donor in deep, confined aquifers. (Simpkins & Parkin, 1993) for instance reported that the methane in a confined aquifer in central Iowa is formed from a high amount of organic matter in the sediment, originating from the burial of a forest during the last glaciation. Also, isotopic analysis show, that the methane and DIC in a confined aquifer in Southern Ontario is formed from old SOM (sedimentary organic matter) rather than from organic matter, leaching from the soil zone (Aravena & Wassenaar, 1993). The formation of methane in deep aquifers is therefore mainly controlled by the amount and reactivity (age) of the organic matter deposited with the sediment.

How high concentrations of methane, that builds up in the water, is controlled, not only by the rate of methane production, but also on the residence time of the water. (Grossman et al. 1989) measured up to 28.8 mM CH_4 in a deep aquifer in Texas, which is about 50 times more than the highest concentration found in the shallow Rømø aquifer.

Another factor, that might influence the concentration of methane in aquifers, is methane oxidation. However, this study has shown, that methane oxidation rates are very low in the transition zone between sulfate and methane in the shallow Rømø aquifer (figure 4.19). A likely explanation is, that the energy yield of methane oxidation with sulfate as electron acceptor is below the threshold level required for the process to occur. The in situ ΔG_r value for the process is approximately 40 kJ/mole. If methane oxidation coupled to sulfate reduction occurs via a reversal of the CO_2 reduction pathway, as proposed by (Hoehler et al. 1994), the reaction might be written as two half reactions:

1) $CH_4 + 3\ H_2O \rightarrow HCO_3^- + H^+ + 4\ H_2$

2) $4\ H_2 + SO_4^{2-} + H^+ \rightarrow HS^- + 4\ H_2O$

Four hydrogen molecules are involved in the reaction, and two bacteria (a methanogenic and a sulfate reducing) has to share the available energy between them. This means, that only 5 KJ/mole H_2 is available to each bacteria, which is below the threshold values reported in the literature for

CO_2 reduction (Schulz & Conrad, 1996; Hoehler, 1998). It is therefore consistent with thermodynamic considerations, that methane oxidation with sulfate as electron acceptor does not occur in the Rømø aquifer.

The low energy yield for methane oxidation with sulfate as electron acceptor is mainly a result of the low concentration of both sulfate and methane in the transition zone. In marine sediments, both methane and sulfate is present in concentrations of up to several mM in the transition zone, whereas their concentrations rarely exceeds 0.1 mM in the transition zone in the Rømø aquifer. Moreover, in marine sediments, methane oxidation seems to stop, when a methane concentration of ~0.2 mM is reached (Whiticar & Faber, 1986). The lack of methane oxidation with sulfate as electron acceptor is therefore also consistent with previous findings from marine sediments. The low concentrations of sulfate and methane are due to a lower input of sulfate than in marine sediments, but also to the lower rates of sulfate reduction and methane production (table 5.1) and to the different modes of transport that dominates (diffusion in marine sediments, advective transport in aquifers).

In deep, confined aquifers, the flow conditions are often very different from shallow aquifers, and it is possible, that the conditions necessary for methane oxidation could be met. Higher concentrations of methane might form due to the long residence time of the water, and if this methane comes in contact with a possible electron acceptor, methane oxidation is likely to occur. One situation, that could lead to such a contact, is formation of methane within layers with a low permeability. If water containing sulfate, nitrate or oxygen flows in more permeable layers, diffusion could transport large amounts of methane into the moving water, where it might act as an electron donor for the reduction of oxygen, nitrate and sulfate in that order.

In contrast, oxygen, nitrate and sulfate will not come near methane containing water in homogeneous deep flow systems, since the redox zones often have a horizontal extension of several kilometres in deep aquifers. Mixture of sulfate and methane containing water by diffusion over such distances will be extremely limited. Accordingly, if the concentration of sulfate in water, infiltrating an aquifer, is raised, it cannot be expected, that methane will become an important electron donor for sulfate reduction in a homogeneous flow system. Rather, sulfate reduction will replace methane production, when high sulfate water enters the previously methanogenic part of the aquifer, as illustrated in (figure 4.8). Since the rate of organic matter decomposition seems to be largely independent of whether sulfate reduction or methane production is the TEAP (table 5.1 and figure 5.3), the amount of sulfate, that the organic matter in the sediment is able to reduce, will be closely related to the amount of methane, that it was able to form, when methane production was the TEAP. A good approximation of a methanogenic aquifer`s ability to reduce sulfate can therefore be obtained, by simply measuring the concentration of methane in the water.

6 Conclusions

The methane in the Rømø aquifer is produced mainly by the CO_2 reduction pathway, but acetate fermentation is a more important process than in deep aquifers. The rate of methane production as well as the relative importance of CO_2 reduction and acetate fermentation varies highly over depth and between sampling locations, despite the apparent homogeneity of the sediment. CO_2 reduction accounts for more than 90 % of the produced methane, where the highest rates are measured, but only 10-80 % elsewhere. The significant contribution of acetate fermentation to methane production results in methane more depleted in deuterium than methane from deep aquifer systems. The rates of methane production are 1-3 orders of magnitude lower than those measured in most marine sediments, but comparable to the rates measured in some lake sediments. These differences reflects the availability of reactive organic matter.

From mass balance considerations it appears, that most of the organic matter being decomposed in redox processes must come from the soil zone, and DOC profiles indicate, that a lot of it must be transported on a colloidal form. Factors likely to influence the load of organic carbon into the aquifer, are the residence time of water in the unsaturated zone and the type of vegetation.

There is a distinct separation between methane production and sulfate reduction. When sulfate enters previously methane producing parts of the aquifer, sulfate reduction replace methane production, whereas methane is readily formed in previously sulfate reducing parts of the aquifer, when sulfate is no longer present. The rate of organic matter decomposition is largely independent of whether sulfate reduction or methane production is the TEAP (Terminal Electron Accepting Process).

Fe(III)-oxides are present everywhere in the aquifer, and Fe(III)-oxide reduction and sulfate reduction occurs concurrently. In sulfate free parts of the aquifer, another redox process, possibly Fe(III)-oxide reduction, occurs concurrently with methane production. This redox process strongly suppress methane production in some sulfate free parts of the aquifer, but not in others. In contrast to marine sediments, methane oxidation coupled to sulfate reduction in the transition zone between sulfate and methane is an insignificant process in the Rømø aquifer. This most likely reflects the much lower concentrations of sulfate and methane.

The hydrogen concentration reflects the dominant TEAP to some degree, but the energy yield for CO_2 reduction at the bulk water hydrogen concentration is so low, that CO_2 reduction must take place within micro niches with higher H_2 concentrations or by interspecies hydrogen transfer between juxtaposed bacteria. The H_2 concentration in the bulk water must therefore be controlled by another redox process, even when CO_2 reduction is the dominant process. In this situation, the

concentration of hydrogen cannot be used to determine the dominant TEAP. The concentration of acetate is completely unrelated to the TEAP. Calculations of the energy yield for acetate turnover show, that suppression of acetate fermentation in parts of the aquifer cannot be due to a low energy yield of the process, which is the traditional explanation. It is suggested, that the concentration of hydrogen controls the turnover of acetate by causing acetogenic methanogens to swich their metabolism from methane production to production of hydrogen from acetate, when the hydrogen concentration is low enough to make this energetically favourable.

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